

CLINICAL AND EXPERIMENTAL STUDIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

with special reference to epidemiology, infection
and in vitro opsonisation of *S. aureus* in serum

Ola Nived



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To KERSTIN, EMILIE and PER

This thesis is based on the following publications, referred to in the text by Roman numerals.

- I Nived O, Sturfelt G, and Wollheim F: Systemic lupus erythematosus in an adult population in southern Sweden. Brit J Rheumatol. Accepted for publication.
- II Sturfelt G and Nived O: Clinical inconsistency, benign course and normal employment rates in unselected systemic lupus erythematosus. Submitted to Clin Exp Rheumatol.
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ABBREVIATIONS

ANA	Antinuclear antibodies
ARA	American Rheumatism Association
C	Complement
CR1	C3b receptor
C1q-BA	C1q binding assay
CRP	C-reactive protein
EDTA	Ethylene diamine tetra-acetic acid
ENA	Extractable nuclear antigen
ESR	Erythrocyte sedimentation rate
Fc-receptor	Receptor for Fc part of immunoglobulin
IC	Immune complex
Ig	Immunoglobulin
nDNA	native deoxyribonucleic acid
RA	Rheumatoid arthritis
RNP	Ribonucleoprotein
S	Serum
SLE	Systemic lupus erythematosus
Sm-antigen	Smith antigen
SS-B	Sjögren's syndrome - antigen B

INTRODUCTION

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

General aspects. Systemic lupus erythematosus (SLE) is an inflammatory multisystem disease of unknown origin, which may affect any system in the body. The wide variety in the clinical manifestations of SLE accounts for its notoriety as a 'mimic' of a number of diseases distributed among almost all fields of clinical medicine (Dubois 1974).

Historically, cutaneous lupus symptoms were commonly thought to be variants of tuberculosis. Hebra characterized the 'butterfly' rash in 1845, and the discoid lesions were described by Cazenave and Chausit in 1852. Kaposi proposed a connection with visceral manifestations in 1872, and Osler published a detailed clinical description of the systemic disease at the end of the 19th century (Cazenave et al., 1875; Osler, 1895). Clinical and pathological studies during the following fifty years (Libman and Sacks, 1924; Baehr et al., 1935; Klemperer et al., 1942) completed the picture of SLE as a disease with poor prognosis, and chiefly affecting women of childbearing age. The cases described were often fulminating with arthritis, cutaneous lesions, serositis, leucopenia, anaemia, nephritis, and neuropsychiatric manifestations. Severe infections, as well as renal and cerebral manifestations, caused the fatalities observed (Jessar et al., 1953). The introduction of such new therapeutic tools, as antibiotics and corticosteroids may have been, at least partly, responsible for the improved prognosis of severe SLE (Ropes 1964; Dubois et al., 1974).

The discovery of the LE cell phenomenon (Hargraves et al., 1948), and the demonstration of antinuclear antibodies (Friou, 1957), gave rise to the modern SLE concept (Dameshek, 1958; Burnet, 1959), the characteristic features of which are the



wide variety of immunological disorders and, in particular, the striking synthesis of autoantibodies. These laboratory discoveries opened up a new clinical era in which milder cases and cases with seemingly disparate clinical pictures could be linked together under the same syndromic category. In parallel classification criteria for SLE evolved (Cohen et al., 1971; Tan et al., 1982).

The causes of SLE are still unknown, but several pathogenetic mechanisms of interest are currently under discussion. Some examples are inherited complement deficiencies (Agnello, 1978), viruses (Pincus, 1982), enzyme deficiencies affecting sex hormone levels (Lahita et al., 1982), and defective genetic control of immune responses (Winchester and Nunez-Roldan, 1982). Much of the search for pathogenetic mechanisms has been carried out with mouse strains developing auto-immune diseases (Steinberg et al., 1982).

Epidemiological aspects. The epidemiological study of SLE was considered infeasible, until the discovery of the LE cell phenomenon in 1948 first provided a hypothetical basis for laboratory screening of suspected cases. Up to now, however, SLE epidemiology has been chiefly based on series of previously diagnosed cases; consequently all published frequencies are probably underestimated (Siegel and Lee, 1973).

The prevalences of SLE found in the middle of the 1950s were about 30 cases per million in Gothenburg (Svanborg and Sölvell, 1957), Malmö (Leonhardt, 1964), and New York City (Siegel et al., 1970), but were as high as 60 cases per million for Malmö and New York at the beginning of the 1960s. The average annual incidence was 10 per million during this period in Malmö and New York (Leonhardt, 1964; Siegel et al., 1970).

Considerably higher frequencies were found in San Francisco between 1965 and 1973, when the extensive records from the comprehensive medical care of a racially mixed population of about 120,000 were screened with reference to the modern con-

cept of SLE and the criteria of the American Rheumatism Association (ARA). Per 100,000 of the population at risk, the incidence was 7.6 cases and the prevalence 51 cases; the prevalence among women aged 15 to 64 was 143, and among black women of the same age group 408 (Fessel, 1974). A very high incidence of SLE in full-blooded Sioux Indians (Morton et al., 1976), and high prevalences for the Oriental and Polynesian populations of Hawaii (Serdula and Rhoady, 1979) illustrate the relevance of well defined ethnic groups in studies of SLE frequencies. No epidemiological studies based on complete medical care of any European population have been published since the adoption of the SLE criteria in 1971.

Prognostic aspects. Within the space of only two decades, the five year survival of patients, reckoned from the diagnosis of SLE, improved from 20 per cent (Jessar et al., 1953) to 90 per cent (Lee et al., 1977). Fatalities due to central nervous system damage fell between 1950 and 1973, possibly in conjunction with the use of high dose corticosteroid treatment, while deaths from infection, vascular complication and malignant neoplasm increased significantly (Dubois et al., 1974). In addition, improvement in the care of uraemia patients during the past decade has resulted in considerably reduced mortality rates for diffuse proliferative glomerulonephritis in SLE, deaths now being largely confined to patients on high dose prednisone (Coplon et al., 1983).

The prolonged duration of disease resulting from improved survival is reflected in a bimodal mortality pattern with late deaths due to atherosclerosis (Urowitz et al., 1976). The overall benefits of corticosteroids treatment in SLE have been questioned (Albert et al., 1979), and although the earlier suggestion (Bulkley and Roberts, 1975) that corticosteroid treatment may accelerate atherosclerosis was later challenged (Haider and Roberts, 1981), the "case for conservative management" based on an increased recognition of mild cases has recently been urged (Hughes, 1982). In all probability, the improved prognosis observable in recent years is, at least

partly, the result of the identification of more such mild cases.

Complicating infections. Already in the pre-steroid era a high frequency of bacterial infections were noted among SLE patients, and infections either caused or contributed to many of the deaths observed (Klemperer et al., 1942). Despite the arrival of antibiotics, problems with bacterial and opportunistic infections in SLE persisted and corticosteroid therapy was identified as one important cause (Estes and Christian, 1971; Lee et al., 1977; Ginzler et al., 1978).

A controlled study of hospitalised SLE patients, compared with rheumatoid arthritis (RA) patients and patients with nephrotic syndrome, showed an increase of disseminated bacterial infections with increasing doses of corticosteroids and decreasing renal function in lupus patients. Moreover, steroid-free SLE patients with normal renal function tended to have higher infection rate than among controls (Staples et al., 1974). A correlation has been found between bacterial infections and the number of disease manifestations per patient (Lee et al., 1977), and a tendency towards increased infection rates with new exacerbations of SLE activity (Ginzler et al., 1978), though in both the Lee and Ginzler series corticosteroid treatment might have accounted for the results. And, as was commented in a recent review of infection in immunologically--mediated disease, "there are still no objective clinical data to support the existence of any relationship between the SLE syndrome and rates of infection" (Cohen, 1983).

Possible factors behind the suggested increase of infections in SLE patients have been listed (Hughes, 1977). Apart from the well-known side effect of corticosteroid treatment (Dale and Petersdorf, 1973), probably mediated by interference with phagocytic receptors (Forslid and Hed, 1982), and the less understood connection with renal failure (Montgomerie et al., 1968), several mechanisms intrinsic to SLE have been proposed. Some examples of such mechanisms are reduced chemotaxis

(Perez et al., 1979; Sturfelt et al., 1983), decreased heat labile opsonic capacity (Jasin et al., 1974), anti-opsonic effect of serum by C1q inhibition of Fc-receptors (Håkansson et al., 1982), reduced monocyte yeast cell phagocytosis in the presence of immune complexes of the cryoglobulin fraction (Svensson et al., 1980), and reduced phagocytic capacity of SLE granulocytes (Brandt and Hedberg, 1969). A possible cell intrinsic factor in SLE could be reduced receptor function, which has so far been observed in conjunction with CR1 receptors of erythrocytes (Miyakawa et al., 1981).

Phagocytic defence and opsonisation. The importance of granulocytes and monocytes, sometimes called "professional phagocytes", for microbial host defence has been recognised for the past hundred years (Metchnikoff, 1883-84), and the anti-bacterial role of neutrophil granulocytes is evident - e.g., in chronic granulomatous disease in childhood (Quie, 1973). The complex process of phagocytosis is initiated by cytotoxins, released from microbes or serum, which chemotactically attract phagocytes. The assembling phagocytic cells identify an opsonised prey and attach it to their cell membranes with the aid of receptors for the Fc-fragment of immunoglobulin G and receptors for activated complement component C3. This sequence subsequently results in ingestion and killing of the prey (for reviews see Stossel, 1975 and Horwitz, 1982). It has been shown that the process of opsonisation or coating of the prey with heat stabile antibodies and heat labile activated complement components, highly promotes the phagocytic process (Wright and Douglas, 1903; Winkelstein, 1973) and thus is one factor determining the rate of phagocytosis (Leijh et al., 1979).

Both in the early "pre-antibody" phase of microbial invasion, and when antibodies are present, opsonisation by the complement system is probably an important defence mechanism (Sterzl, 1963; Williams and Quie, 1971; Stossel, 1975). The hypocomplementaemia observed in active SLE (Vaughan et al., 1951), especially in patients with nephritis (Lloyd and Schur,

1981; Sturfelt et al., 1984), could be linked to reduced opsonic capacity and defective granulocyte phagocytosis in SLE sera (Jasin et al., 1974). However, when monocyte bactericidal activity in the presence of SLE sera was studied, no relationship was found between bactericidal activity and complement (Nived et al., 1980), but both the Jasin and Nived series suggested a relationship between reduced serum-dependent phagocytic killing and retrospective clinical infections (Nived et al., 1980) or current ones (Jasin et al., 1974). However, the critical importance of the serum concentration for opsonisation, that has since been reported (Tofte et al., 1980) renders the interpretation of these earlier results more difficult, as their validity with undiluted serum is uncertain.

THE PRESENT INVESTIGATION

The aims of the present clinical study (Part one) were:

- To investigate SLE incidence, prevalence and mortality in a defined Swedish population (I).
- To describe the clinical features of unselected SLE patients and to follow prospectively the course of these patients for two years (II).
- To observe, in a prospective and controlled study, the frequency and spectrum of infections occurring in unselected patients with SLE, compared with matched controls and with matched RA patients during 6 months (III).
- To follow prospectively a large group of selected and unselected SLE patients for a period of 30 months, to test the hypothesis of an SLE disease activity related increase of infection rates (III).

The aims of the present experimental study (Part two) were:

- To develop an in vitro assay for the study of bacterial opsonisation in undiluted sera (IV).
- To relate opsonisation of S.aureus in SLE sera to disease manifestations and complicating infections (IV).
- To analyse possible mechanisms behind observed variations in the capacity of SLE sera to opsonize S.aureus (IV).

PART ONE: CLINICAL STUDIES

IN SLE (I, II, III)

THE SLE CLINIC IN LUND

To improve monitoring of SLE patients and to facilitate scientific studies of SLE, an SLE clinic was organised within the Department of Rheumatology at the University Hospital of Lund in December 1980. At its inception, about 45 patients already diagnosed as having SLE and attending the regular out-patient clinic were transferred to the new unit. Subsequently, all patients referred to the Department of Rheumatology with a known or suspected SLE diagnosis have been assessed by either of two doctors at the clinic.

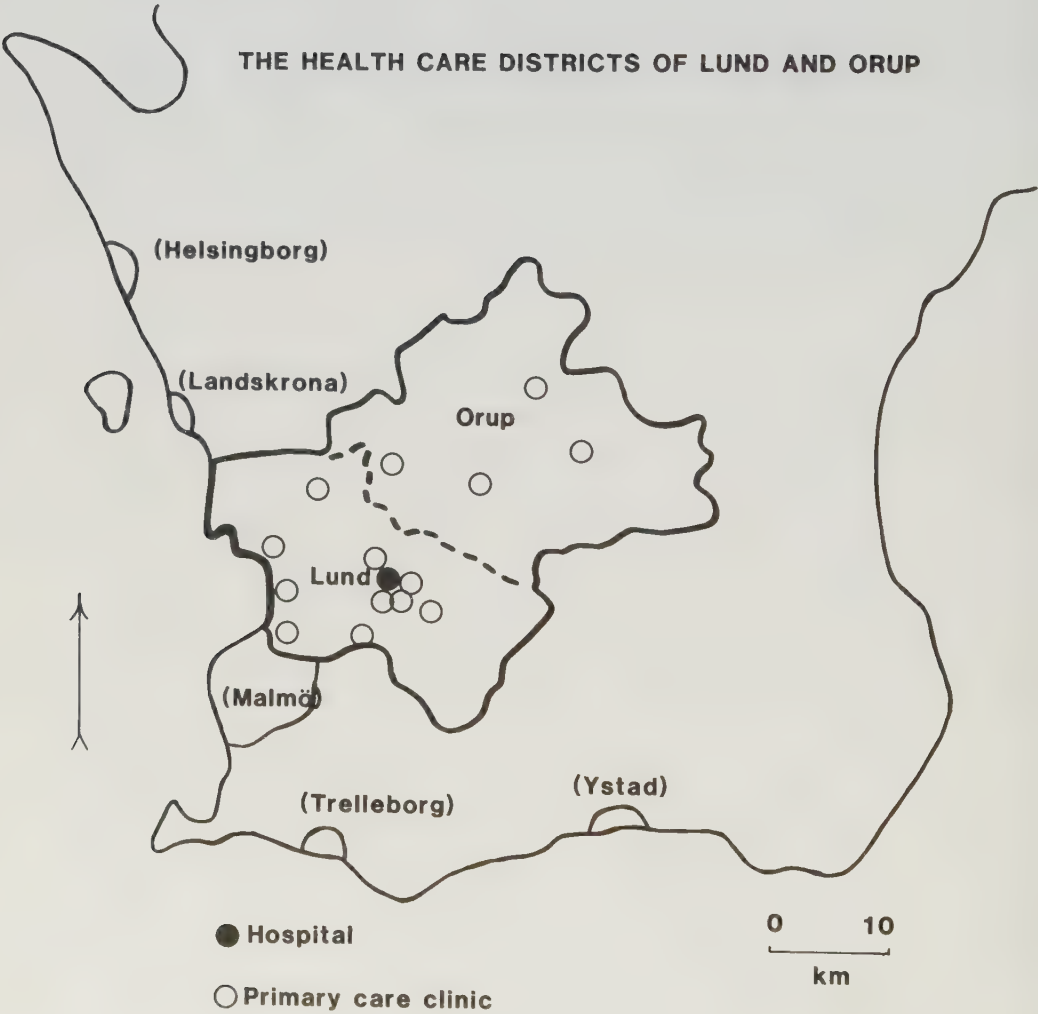
A 'definite' SLE diagnosis is required for patients followed at the clinic and is considered acceptable in patients exhibiting, serially or simultaneously, multisystem disease and immunological disturbances without any other possible diagnosis, chiefly according to the principles proposed by Fries and Holman (1975). However, neither ANA-positivity, nor the presence of antibodies to DNA are prerequisites (Maddison, 1982). Once diagnosed, patients are classified according to ARA criteria (Cohen et al., 1971; Tan et al., 1982). It was considered important to continuously re-evaluate the SLE diagnosis.

At the SLE clinic, definite cases of SLE, as defined above, are prospectively and sequentially followed at regular intervals. When required, specially trained physicians at the Departments of Infectious Diseases, Neurology, Psychiatry or Nephrology are consulted. So far (1984), about 150 patients have been seen for an aggregate of about 1200 visits and 95 patients are currently being seen at regular intervals.

The SLE clinic provided the basis for the present investigation and the clinical and laboratory data obtained were computerised, stored and handled using specially designed programmes in BASIC (Facit desk top computer).

Fig. 1

SCANIA



PATIENTS AND METHODS

Population at risk (I). In the health care districts of Lund and Örup in Scania (Fig.1), a province in southern Sweden populated almost exclusively by caucasians, the population is about 200,000, of which about 157,000 are over 14 years of age (for more detailed figures, see original articles). The area is served by one hospital, the University Hospital of Lund, 14 primary care clinics and a few private clinics. Computerised diagnosis registers have been built up since 1968 and now (1984) cover the hospital and all primary care clinics of the districts, with the last three primary clinics having been added in 1984. SLE epidemiology in this defined population was studied during the years 1981 and 1982.

Retrieval of unselected patients (I, II, III). The social security numbers of all patients with a diagnosis of SLE (WHO number 734.10) during the years 1973-1980 were extracted from the computerised diagnosis registers. Outpatients records, and registers of ANA positivity in the laboratory serving the area, were also screened for possible cases. During the period under consideration, 210 individuals over the age of 14 with a diagnosis of SLE were located, of which 71 were members of the population at risk, the remaining 139 having been patients referred to the University Hospital from other parts of southern Sweden. Of the 71 local patients with an SLE diagnosis, 8 had died before the start of the study on January 1, 1981, leaving 63 to be considered for inclusion in the group to be studied.

In 14 cases, the diagnosis of SLE was changed at the lupus clinic, when clinical findings were considered insufficient or rendered another diagnosis more probable. Thus, 6 patients were classified as possible SLE cases, 7 patients were diagnosed as having erosive seropositive rheumatoid arthritis with Sjögren's syndrome, and one patient had psoriatic arthritis in combination with myelofibrosis. In addition the files of 119 patients with other multisystem diseases (specified, n=55; or

unspecified, n=64) were screened for possible SLE cases, but none of the 23 patients thus traced and considered as possible SLE cases received an SLE diagnosis at the clinic.

Patient retrieval was cross-checked for completeness after the study period, firstly by personal communication with physicians practising in the area and secondly by the means of a separate computer register at the Dalby district health care clinic. The personal communications revealed one more patient with definite SLE within the area, whereas the entire registers of one primary care district did not disclose any more diagnosed cases of SLE.

To sum up, from the population under consideration, 50 cases with a definite diagnosis of SLE, of patients living at the start of the prospective study, were obtained by retrieval; 15 new definite cases were diagnosed during the study, bringing the total of the group studied up to 65 patients by prevalence testing time on December 31st, 1982. With the exception of two who declined to participate but who had been seen earlier, all patients were followed at the University Hospital, 43 sequentially at the SLE clinic, and the remaining 20 attending the clinic at least once. (For detailed information of sex and age distribution see original articles.)

Selected patients (II, III). A parallel group was established of 42 patients with a definite SLE diagnosis of which 39 were sequentially seen in the SLE clinic. These patients were referred from other parts of southern Sweden than the health care districts of Lund and Örup. When non-epidemiological questions were under consideration, selected and unselected SLE patients followed were handled as one group of 102 patients.

Rheumatoid arthritis (RA) patients (III). In order to study the infection pattern of SLE patients in a controlled manner one RA and one control group were matched against the epidemiological SLE group. Sixty RA patients, including 41 with

classical RA, 16 with definite RA according to ARA criteria (Ropes et al., 1958), and 3 adults with juvenile onset of rheumatoid factor positive polyarthritis were matched with regard to sex, age and corticosteroid dosage. The patients were followed for six months in our specialised out-patient unit for chronic arthritis.

Controls (III). Sixty out of 75 healthy controls (husbands or wives of patients at the Department of Rheumatology) agreed to participate in a prospective study. They were matched against the epidemiological SLE group with regard to age and sex and completed a questionnaire sent out after the observation period of six months.

SLE-monitoring (II, III). At each clinical attendance, organic manifestations and disease activity were assessed without knowledge of immunological test results. The patients were assigned to clinical subsets according to the principles proposed by Lightfoot and Hughes (1976), as modified by Sturfelt (Thesis, 1984); subsets were thus based on symptoms during the observation period.

Manifestations of active disease occurring during the study were divided into minor and major categories as previously described (Sturfelt, Thesis, 1984). In short, minor manifestations (usually arthritis in combination with skin manifestations) would be safely treated with antimalarial drugs and/or low dosages of Prednisolone (<20 mg/day). On the other hand, major manifestations always warranted hospitalisation and treatment with Prednisolone at dosages of more than 20 mg/day, sometimes in combination with other immunosuppressive drugs.

Infection registration (III). Infections suspected clinically were confirmed by positive cultures, and by radiographic evidence or prompt regress in response to antibiotic therapy. Viral infection was suspected when patients presented at the clinic with acute incipient pharyngitis, rhinitis or gastroenteritis, and bacterial cultures were negative. Organisms were

designated as opportunistic in accordance with Cohen's listing (Cohen et al., 1982), which is based on principles proposed by von Graevenitz (1977). The infection studies were performed during the period from December 1980 to June 1983.

SLE laboratory routines (II, III). At each clinical attendance urine was analysed and blood sampled for routine analysis of S-Hb, leucocytes, platelets, erythrocyte sedimentation rate (ESR), S-orosomucoid, liver enzymes, and S-creatinine. Complement components C1q, C4 and C3 were measured immunochemically (Sjöholm, 1975), C $\bar{\text{T}}$ r-C $\bar{\text{T}}$ s-C $\bar{\text{T}}$ inactivator complexes by electroimmunoassay (Laurell et al., 1979), and C-reactive protein (CRP) was analysed immunochemically whenever infection was suspected. Serum and EDTA plasma (5 mmol EDTA/l) were collected in aliquots and stored at -80°C for further analysis if necessary.

Epidemiological estimates (I). The mean annual incidence of SLE was calculated as the mean number of new clinically diagnosed cases per 100,000 mean population at risk during the years 1981 and 1982. The prevalence of SLE was calculated as all clinically diagnosed cases per 100,000 population at risk who were living on December 31, 1982. The mean annual mortality rate was calculated as the mean number of deaths among clinically diagnosed cases of SLE during the years 1981 and 1982. In all estimates only those over 14 years of age were considered.

Intra-SLE group matching (III). To study the influence of disease activity and flares on the number of infected patients, the patients were used as their own controls, periods during SLE activity being matched with inactive periods of equal length, and periods before and after flares were similarly matched. Periods of inactive SLE were used to study the effects of corticosteroid treatment, patients being stratified to groups with or without corticosteroid treatment. Patients with periods of leucopenia or hypocomplementaemia were similarly stratified (for details, see original paper).

RESULTS

Epidemiology (I)

Fifteen new cases of definite SLE were clinically diagnosed in the health care districts of Lund and Örup during the years 1981 and 1982. The calculated incidence for the total group was 4.8 cases, for all females 7.6 cases, for females in the 45-64 age group 15.9 cases, and for males 2.0 cases (all figures per 100,000 population at risk).

Fourteen new cases of SLE were retrospectively diagnosed in the area during the years 1979 and 1980 (unpublished observation).

Sixty-one patients with a definite clinical diagnosis of SLE were living in the epidemiological area on December 31, 1982. The calculated prevalence for the total group was 38.9 cases, for all females 64.8 cases, for females in the 45-64 age group 99.2 cases, and for males 11.7 cases (all figures per 100,000 population at risk).

Four patients with a definite clinical diagnosis of SLE, residing in the epidemiological area, died during the years 1981 and 1982. All were elderly women (63-79 years) with varying disease duration (2-30 years). The fatalities were caused by myocardial infarction complicated by bronchopneumonia, oral carcinoma, sepsis and cerebro-vascular disease with bronchopneumonia. The mean annual mortality was 1.3 cases per 100,000 population at risk.

Retrospectively, 3 fatal cases of SLE occurred in the area during the years 1979 and 1980 (unpublished observation).

Clinical features and course in unselected SLE (I, II)

Sensitivities were 92 per cent according to the 1971 ARA criteria (Cohen et al., 1971), and 94 per cent according to

the 1982 ARA criteria (Tan et al., 1982) (for distribution, see original papers). Males fulfilled more internal organ criteria than females ($p < 0.03$), but tended to fulfill less cutaneous criteria ($p < 0.09$).

Among cumulative findings, relatively high frequencies of gastro-intestinal manifestations (17%) and pulmonary vascular diseases (14%) were noted. Nephritis was diagnosed in 17 patients. Sjögren's syndrome was present in 13 of the female patients with objective confirmation in 10 cases. Only 10 per cent of all pregnancies, by history or observed, ended with spontaneous abortion, a figure comparable to that found among Swedish pregnancies in general.

Forty-four per cent of the patients were hospitalised during the years 1981 and 1982, with a median duration of 8 days for each hospital admission. Thirty-two patients were on corticosteroid therapy during 1981 and 1982, with a mean daily dosage of 7.5 mg prednisolone. Seventeen patients were treated with oxychloroquine and 8 with azathioprin. Forty-three of 54 patients below retirement age were employed, a proportion comparable to that in the general Swedish population, but figures for sickness absence were higher in the SLE group ($p < 0.004$).

Major flares were more common during summer ($p < 0.05$), and in conjunction with 5 flares occurring in winter possible triggering factors were found such as exposure to UV light, cessation of therapy or preceding infection. A change in clinical picture between sequentially observed flares occurred in 10 of 16 patients with major flares and in 4 of 13 patients with minor flares. Clinical disease activity increased ($p < 0.01$) the concentrations of ESR, S-CRP, S-orosomukoid and C₁r-C₁s-C₁ IA complexes indicating C1 activation.

Infections (III)

A total of 240 infections, of which 163 occurred in the epidemiological group, were recorded in 79 of the SLE patients.

Of the infections, 108 were bacterial, 12 were fungal, and 120 were suspected to be viral.

Controlled study of matched groups. Bacterial infections, other than those of the lower urinary tract, occurred in 3 controls, 6 RA patients (n.s.) and 15 SLE patients ($p < 0.01$ versus controls, $p < 0.06$ versus RA). Opportunistic infections were found in 1 control, 1 RA patient and 5 SLE patients of the matched groups (n.s.). Since the data both for urinary tract infections and for suspected viral infections were comparable in all three groups, no further study was devoted to those types of infection.

S.aureus infections. Of 23 S. aureus infections among 16 SLE patients, 17 occurred in the epidemiological group (4 of which during the six month controlled study), while none occurred in controls or RA patients. Eighteen S. aureus infections were cutaneous, nine of which were preceded by verified injury or stress causing rupture of the skin.

Influence of immunosuppressive treatment. Of patients with bacterial infections, the number of those on corticosteroid treatment (19/48 over an aggregate 849 months) did not significantly exceed the number of those not on corticosteroids (11/44 over an aggregate 849 months). There was a tendency for the number of patients with bacterial infection, and of those with opportunistic infection, to increase with increased steroid dosage (for complete data see original paper).

Previous history of prolonged corticosteroid therapy was more common among 10 SLE patients with more than 2 bacterial infections than among 50 SLE patients without bacterial infections ($p < 0.05$), though actual corticosteroid dosage and lupus activity were comparable. Moreover, the multi-infected patients belonged more often to a vascular SLE subset ($p < 0.01$).

Influence of clinical SLE activity. Among those serving as their own controls (cf methods), 5 of 22 SLE patients without

corticosteroid treatment contracted bacterial infections during disease activity, and none of the 22 became infected during inactivity ($p < 0.04$). Thirteen of 41 SLE patients on corticosteroids had bacterial infections during active disease but none of the 41 were infected during inactive SLE ($p < 0.001$). Regarding opportunistic infections, only the corticosteroid group showed an increase in the number of infected patients ($p < 0.01$).

Of 49 SLE patients with flares of disease activity, one had a bacterial infection within 2 months before flare and 9 others had bacterial infections within 2 months after flare, whereas 39 had no bacterial infections in conjunction with flare. The increase of bacterial infections observed after flare was significant ($p < 0.03$). Frequencies of suspected viral infections before and after flare were comparable.

Eight bacterial infections were noted during an aggregate 55 months in patients with vascular manifestations from the skin, and three such infections were recorded during an aggregate 20 months of observation of uraemia patients. Opportunistic infections tended to be more frequent in conjunction with leucopenia and hypocomplementaemia.

Hospital admittance due to infection. In the epidemiological group 12/28 hospital admissions were partly due to infection. Among the 81 sequentially followed patients, 24/56 hospitalisations were due to a preliminary diagnosis of infection which was subsequently verified in 10/24 cases. Infection was accompanied by initial fever in 6/17 cases, and neither prednisolone therapy nor laboratory findings such as ESR, S-CRP and leucocyte counts distinguished patients with infections later verified from those without infection.

Mortality due to infections. Of a total of seven deaths, infections caused or contributed to five, namely sepsis in three cases (1 *S. aureus*, 1 *E. coli* and 1 gram-negative), and bronchopneumonia in two cases.

DISCUSSION

The present work is the first clinical study of unselected SLE patients from a defined European population since the adoption of the ARA criteria in 1971, and the first controlled study of infection in SLE patients based on an epidemiological group. The accessibility of hospital records in the only hospital serving the population at risk, the computerised diagnosis registers covering the area, and reliability of population statistics ensured that retrieval of diagnosed SLE cases was satisfactorily complete. This is borne out by the finding of only one additional patient after exhaustive cross-checking. Another factor promoting location of diagnosed SLE-patients in the area has been the close cooperation between local doctors and the SLE clinic. Since, however, like all earlier epidemiological studies of SLE, the present one was chiefly based upon cases already diagnosed, our figures for incidence, prevalence and mortality may in fact be underestimations (cf Siegel and Lee, 1973).

The framework of the SLE clinic with regular patient attendance has facilitated diagnostic activities and made sequential studies possible. One indication of diagnostic accuracy is the bacterial and viral infection rates, which were, respectively, three and two times those reported by Ginzler et al. (1978). Overestimations, on the other hand, were avoided by recording only those infections detected clinically and confirmed by culture, or those the existence of which could be inferred from radiographic findings and prompt regress in response to antibiotic therapy.

In the present investigation (I), both incidence and prevalence exceed by 5 to 10 times those found in the same area 20 years ago by Leonhardt (1964). Probably this increased recognition of patients with SLE is partly a consequence of the introduction of classification criteria, paired with increasing professional knowledge of the disease. Since incidence figures in this series (I) exceed the mortality rate, clearly

the group still is expanding (cf Siegel and Lee, 1973). Furthermore, retrospective incidence and mortality two years before the present investigation, are of the same order as the figures published (see Paper I). Thus an increasing prevalence of SLE in the near future seems most likely, but whether this will reflect an increase in the disease itself or changing diagnostic concepts cannot be determined at present.

Comparison of our epidemiological data with Fessel's (1974) in San Francisco a decade earlier, indicates a somewhat lower incidence and prevalence of diagnosed SLE in Scania than in California. This discrepancy is most probably due to the presence of a mixed population in San Francisco. In addition to the defined black population, undefined indian, oriental, mexican, polynesian and chinese ethnic groups are also included in Fessel's study, all with demonstrably or suspected higher frequencies of SLE (Fessel, 1974; Morton et al., 1976; Serdula and Rhoady, 1979; Frank, 1980; Hart et al., 1983). However, since a chiefly white population in Dunedin, New Zealand, had much lower prevalence of SLE (Meddings and Grennan, 1980) than our population, it is by no means certain that race is the only frequency-determining factor in SLE.

A recent report of SLE prevalence in Finland of 28 cases per 100,000 population was based only on hospitalised cases (Helve 1984), and is in agreement with our corresponding figure (25/100,000) based on hospitalised cases over a 10 year period retrospectively observed (I).

With the increasing recognition of SLE, more mild cases have been found (Hughes, 1982). This is reflected in the high age of most fatal cases (I, II), the high median age in the epidemiological group (I), the infrequent hospitalisation (I), the low overall level of corticosteroid treatment (II, III) and the high employment rate (II). Interestingly, high age has been reported among SLE patients in a white population before, namely in Rochester, Minnesota, during the 1960s (Kurland et al., 1969). Since, however, RA patients with positive LE cells

were included in that study, interpretation is difficult. In contrast to SLE abortion materials presented earlier (Estes and Larson, 1965; Fraga et al., 1974; Grigor et al., 1977), the low frequency of spontaneous abortion in our epidemiological group might be another consequence of the mild disease.

Classification of our patients according to ARA criteria (Cohen et al., 1971; Tan et al., 1982) also illustrates the benign character of the disease in the group. Thus, fewer renal and neurological criteria were fulfilled than in most other series (Dubois et al., 1974; Estes and Christian, 1971; Lee et al., 1977; Grigor et al., 1978); nevertheless, high sensitivities of the criteria (92% and 94 %, respectively) were found, in agreement with Canoso and Cohen (1979) and Levin et al. (1984). On the other hand, pulmonary vascular and gastro-intestinal manifestations, not included among the criteria, were common findings, perhaps due to the availability of good non-invasive diagnostic facilities.

Our observation of a different clinical picture in men, at least as measured by fulfilled organic criteria and perhaps also by cutaneous criteria (I), contrasts with earlier findings in selected SLE (Miller et al., 1983) and needs confirmation in an epidemiological study with more men. However, our male prevalence data are comparable to those found among non-blacks in Fessel's study (1974), contrasting with the difference between our female prevalence and the non-black female prevalence in San Francisco. One possible explanation for this discrepancy might be a more complete identification in the present series of 'one shot' male SLE, clinically differing from female SLE.

Sjögren's syndrome (SS) is recognised today as accompanying SLE more commonly than was previously suspected (I,II, Alarcon-Segovia et al., 1974). However, the discrepancy between the frequencies of clinical SS and the high frequencies of laboratory tests compatible with SS, probably indicates a mild and fluctuating character of this companion disorder in

patients with SLE. The clinical and serological features of SLE and SS are in fact rather similar, but differences also exist, both clinically and in autoantibody patterns (Moutsopoulos et al., 1980). In view of the similarities, it is not surprising that differential diagnostic problems sometimes appear. Obviously misdiagnosed cases, such as ANA positive RA patients with Sjögren's syndrome having been diagnosed as SLE, were common (I).

Bacterial infections, other than those of the lower urinary tract, were more common in SLE than in controls ($p < 0.01$), but barely more common than in RA ($p < 0.06$). This tendency indicates a steroid-independent increase in bacterial infections, as RA patients also were matched with regard to corticosteroid dosage. Opportunistic infections, mostly fungal, and suspected viral infections were comparable in the three matched groups (III).

An SLE disease-related susceptibility to infection is clearly indicated by our finding among steroid-free subjects that more patients contracted bacterial infections during active SLE than during periods of disease quiescence ($p < 0.04$). Further evidence of such a relationship between SLE and bacterial infection was our finding that patient infections were more frequent after flare than before ($p < 0.03$). This predisposition for bacterial infections in SLE has never been shown before in a controlled manner (Cohen, 1983), although it has been suspected (Staples et al., 1974; Lee et al., 1977; Ginzler et al., 1978).

An intrinsically high frequency of opportunistic and viral infection in SLE has also been proposed (Ginzler et al., 1978), though the supporting data might equally well be interpreted as indicating a corticosteroid relationship. Apparently the normal frequency of lower urinary tract infections has not been reported before in SLE, though it has been described in RA (Mowat et al., 1970). In conclusion, our data support a predisposition, intrinsic to SLE, for bacterial

infection, other than lower urinary tract infections, which is positively correlated to disease activity. However, infections do not generally precede flares, and only rarely do they trigger disease activity. This trigger hypothesis, originating from Shearn and Pirofsky (1952), and proposed again only recently (Cohen, 1983) has previously been systematically refuted (Ginzler et al., 1978).

The relatively high frequency of infections with common pathogens, especially of *S. aureus*, in the present investigation suggests defective phagocytic defence in SLE (Armstrong, 1974). The high *S. aureus* incidence, noted already in our pilot study (Nived et al., 1980), is also probably dependent on the occurrence of skin trauma (van Graevenitz, 1977), which was found in half of our cases. Since, moreover, an increase of *S. aureus* sepsis and endocarditis in conjunction with chronic disease has recently been noted among patients from the same region (Hedström and Christensson, 1983), our results might also have been subject to such microbiological variation.

Although the low overall corticosteroid treatment (III) probably prevented the detection of any connection between corticosteroid dosage and infection in the present series, there was a tendency towards increase in the frequency of patients infected with opportunistic organisms with increasing steroid doses. At all events, the major importance of immunosuppressive treatment for infections in SLE has been convincingly demonstrated in earlier work (Staples et al., 1974; Lee et al., 1977; Ginzler et al., 1978). Less well-known is the possible connection, suggested by our retrospective data, between earlier long-term corticosteroid therapy, SLE with vascular manifestations in the skin, and patients with recurrent bacterial infections. Although this relationship requires confirmation by prospective study, it would appear to reflect an iatrogenic effect, possibly similar to the hypothesised acceleration of atherosclerosis in steroid-treated SLE (Bulkley and Roberts, 1975; Urowitz et al., 1976).

Not only is a correct diagnosis of infection in an acutely ill SLE patient extremely difficult, but the present series have shown that disease activity and infection are not infrequently present simultaneously, indicating that two diagnoses might often be more appropriate than one. This dilemma is further compounded by the fact that a wrong single therapy with corticosteroids or antibiotics, might have a severely deleterious effect on an SLE patient in a critical situation. The guidance provided by laboratory findings other than positive bacterial cultures, such as S-CRP, leucocyte counts and ESR appears to be minimal (III), despite some reports to the contrary (Hill, 1951; Pepys et al., 1982), but in agreement with others (Zein et al., 1979; Nived and Sturfelt, 1982).

PART TWO: EXPERIMENTAL STUDIES IN SLE (IV)

In view of the recognised anti-bacterial role of phagocytes (Quie, 1973), it was natural to suspect an SLE inherent defect of phagocytic defence as one possible mechanism contributing to the high frequency of bacterial infections observed in SLE. Jasin and co-workers (1974) had shown that reduced opsonic capacity, leading to reduced efficacy of the phagocytic system, correlated with hypocomplementaemia in SLE. However, in our own pilot study (Nived et al., 1980), *S. aureus* killing by monocytes bore no relationship to complement concentrations, which suggested that additional mechanisms might be of importance. The pronounced impact of such kinetic factors as time and concentration having been reported in the meantime (Tofte et al., 1980), the aim of the present investigation was firstly to develop an in vitro assay with undiluted serum for opsonic studies, secondly to apply the method on SLE sera to study possible connections with clinical findings, and thirdly to analyse mechanisms behind variations of opsonic capacity detected in serum with the present method.

PATIENTS AND METHODS

Patients. Forty-one consecutive patients, from the sequential study of SLE at the SLE clinic, were included in the present study. All fulfilled at least four of the 1971 (Cohen et al.) or of the 1982 (Tan et al.) ARA criteria for SLE. Median age was 37 years and 4 patients were males.

Serum. Blood was sampled as described earlier (cf p. 15). Serum collected at room temperature and at 37 °C, and EDTA plasma (5 mmol EDTA/l), were divided in aliquots and stored at -80 °C. The sera handled at room temperature were used for complement analysis and the sera handled at 37 °C were used for opsonic studies and cryoglobulin isolation. Fifty-nine sera (the first available sample from each patient during the prospective study together with further samples from some patients) were analysed with the opsonic assay (for details see original paper).

Pooled serum was collected from 34 healthy volunteers. Individual sera were collected from 15 healthy members of the hospital staff, 7 of the sera being used for determination of the variance of single sera from one subject to another (Hoffman and Bullock, 1973).

Serum from one patient in a family with fulminant meningococcal infections and hereditary properdin deficiency (Sjöholm et al., 1982) was kindly provided by Dr J.H. Braconier, Department of Infectious Diseases, University Hospital, Lund.

Phagocytes. 100 ml blood was drawn from a cubital vein in healthy members of the hospital staff. In all, 21 separate blood-donors have contributed and 9 of these (8 women and 1 man) have each contributed on more than 5 occasions. No present infection or medication was accepted at the time of blood collection. The blood was immediately defibrinated by gentle rotation for 10 minutes in a flask containing glass

beads. In some experiments heparinized blood, EDTA blood and defibrinated blood was studied simultaneously, always with similar results.

Granulocytes and monocytes were isolated with a modified Boyum technique (Böyum, 1968) using Dextran T 500 (Pharmacia, Uppsala, Sweden) sedimentation, Isopaque-Ficoll (Lymphoprep, Nyegaard and Co., Oslo, Norway) centrifugation, in two steps for monocyte preparation, and for erythrocyte lysis, of the granulocyte portion, 0.87 per cent ammonium-chloride was used. In seven isolations the monocyte fraction was visualised with esterase staining according to Yam (1971), and constituted 20.5 per cent (range 13-33) of the mononuclear leucocytes. Neutrophil contamination of the mononuclear fraction was 1.1 per cent (range 0-3) and mononuclear contamination of the granulocyte fraction was 1.8 per cent (range 0.5-3.5).

Bacteria. *Staphylococcus aureus* (Strain 502 A) was used as the test organism. In some experiments, separately reported under Results, another *S. aureus* strain (Wood 46) and a clinical isolate of *E. coli* (from a lower urinary tract infection, kindly provided by Dr. Göran Kronvall, Institute of Medical Microbiology, University of Lund) were run in parallel. Bacteria were cultured overnight in Bacto Antibiotic Medium 3 (Difco, Detroit, USA), twice washed in isotonic sodium chloride and then opsonised.

Opsonisation. The washed bacterial pellet was suspended in 1 ml undiluted patient or pooled control serum and incubated for 3 min at 37 °C. In some experiments (noted in Results) 0.1 ml sera diluted in 0.9 ml Parker 199 (National Bacteriological Laboratory, Stockholm, Sweden) was used, and in some (noted in Results) 30 min incubation was used. After arrest of the opsonisation by 0.01 M EDTA and washing, the bacterial concentration was adjusted.

Bacterial killing. Eight million bacteria per ml were incubated with a modified Maaloe technique (Maaloe 1946) at 37°C

with 5 million phagocytes per ml, the incubation volume used being 0.25 ml. The incubation medium, Parker 199, contained 10 per cent heat inactivated normal pooled sera to support phagocytes. After 15 min incubation was arrested with distilled water containing 0.01 per cent bovine serum albumin and 1.5 per cent of the detergent Triton X 100 at 4 °C for 3 min to permit cellular lysis in the tube. After dilution, viable bacteria were determined by colony counting using the pour plate method. All incubations were performed in duplicate, controls without cells were always included, and no deaths occurred.

Bacterial killing was not supported by opsonisation in heat-inactivated normal pooled sera or in EDTA sera. Intracellular killing, as determined by two step incubation with lysostaphin (Schwarz/Mann, Orangeburg, N.Y., USA) added between the incubations, was found to be of the same range independent of the opsonic source, SLE sera or normal pooled sera (Not published). Prednisolone (Precortalone aquosum, Organon, Holland) 1 ug/ml or 20 ug/ml added to serum before opsonisation, did not affect opsonisation. The rate of killing was linear for 60 min, when plotted on a lin-log scale.

Fixation of C3. Anti-C3d rabbit IgG (Anti-human C3d complement, Dakopatts A/S, Copenhagen, Denmark) was labelled with 125-I (Amersham, Buckinghamshire, England) using the chloramine-T method (McConahey and Dixon, 1966). After the arrest of opsonisation by adding 10 ml 4 °C isotone saline, washing of bacteria and dilution in Parker 199, labelling with anti-C3d was performed (for details, see original article). The radioactivity in the bacterial cell pellet was measured after washing (Gamma Sample Counter, LKB, Stockholm, Sweden). The results were expressed as the percentage of cpm in reference (pooled) sera. With heat inactivation of sera (pooled or SLE sera) or with sera from a patient with hereditary C2 deficiency, cpm was reduced by more than 80 per cent.

Complement components. The complement components (C) C1q, C1s, C3 and C4 were measured in all sera by electroimmunoassay

(Sjöholm, 1975; Laurell et al., 1978). All sera were screened with a haemolysis in gel functional complement assay for deficiencies in the classical and alternative pathways of complement (Truedsson et al., 1981).

Circulating immune complexes. Circulating immune complexes were measured using a fluid phase C1q binding assay (C1qBA) as described by Zubler et al. (1976). The results of C1qBA measurement in 9 SLE sera collected both at room temperature and 37 °C were comparable for the two temperatures.

Cryoprecipitation. Cryoprecipitation was performed according to Svensson et al. (1980), with storage at 4 °C for 4 days as suggested by Cream (1972). Native SLE serum, supernatant of SLE serum after elimination of cryoprecipitate, and pooled serum with dissolved cryoglobulin were prepared from 9 SLE patient sera. Five of the patients had reduced opsonic capacity ($p < 0.05$) (for details, see original paper).

RESULTS

Development of the assay

Bacterial opsonisation in undiluted normal pooled serum for 3 or 30 min produced comparable phagocytic killing. On the other hand, killing was lower when 3 min opsonisation in 10 per cent normal pooled serum was compared with 30 min opsonisation (monocytes $p < 0.01$, granulocytes $p < 0.01$). Furthermore, opsonisation in 10 per cent serum was less effective than opsonisation in undiluted sera, both when 3 min (monocytes $p < 0.001$, granulocytes $p < 0.001$) and 30 min (monocytes $p < 0.05$, granulocytes $p < 0.01$) opsonisation times were compared. The observed killing capacities of the two phagocytic cell systems were comparable. Opsonic capacities were significantly reduced in sera from 2 SLE patients with hereditary C2 deficiency ($p < 0.001$), and in one serum sample from a patient with pronounced hypocomplementaemia, probably genetically determined, of C4 ($p < 0.05$). Opsonisation in sera from a patient with inherited properdin deficiency, on the other hand, was within the normal range.

Opsonisation in sera from SLE patients

Opsonic capacity was reduced ($p < 0.05$) in sera from 20 of 41 SLE patients. Granulocyte and monocyte killing of bacteria opsonised in the same 22 SLE sera correlated ($r = 0.68$, $p < 0.001$).

In 11 of 14 sera, collected either during disease activity or during the four months preceding a flare, opsonic capacity was reduced, as compared with 6 of 27 sera sampled during quiescent disease ($p < 0.004$).

A correlation existed between the number of active organ systems and the reduction of opsonic capacity ($r=0.82$, $p<0.001$).

In 8 patients, the opsonic capacities of their sera were reduced in active disease, as compared with those of their sera in quiescent disease ($p<0.05$).

Opsonic capacity was reduced in 4 out of 5 sera sampled within a 2 month period preceding bacterial infection, as compared with 9 out of the remaining 36 sera ($p<0.05$).

In 6 patients, sera were collected during infection but before antibiotic treatment was started, and in all 6 sera opsonic capacity was within the normal range.

Opsono-phagocytic killing of *E. coli* was reduced in one of 15 SLE sera. Nine of these sera had reduced opsonic capacity of *S. aureus*. However, when the results were ranked and compared, a correlation, chiefly within the normal range for *E. coli*, was found ($r=0.68$, $p<0.01$; unpublished observation).

One of 5 sera sampled within 2 months before an *E. coli* infection, of the lower urinary tract, showed reduced opsono-phagocytic killing of *E. coli* (unpublished observation).

Mechanisms of reduced opsonic capacity

The immunochemically determined concentrations of complement components and the functional complement assays showed no significant differences between sera with normal opsonic capacity and sera with reduced opsonic capacity, nor was there any difference in corticosteroid treatment.

The bacteria-associated C3d, determined by labelled antibodies, showed no disparity between 9 sera with reduced opsonic capacities and 5 sera with normal opsonisation. The observed C3 concentrations showed no correlation with opsonic capacity determined by granulocyte killing of *S. aureus* ($r=-0.04$).

Staphylococcus aureus 502 A and *S. aureus* Wood 46 were opsonised in parallel in 6 SLE-sera to study the importance of staphylococcal protein A for opsonisation. Five sera showed reduced opsonic capacities ($p < 0.05$) for *S. aureus* 502 A, whereas values for the opsonisation of *S. aureus* Wood 46 were within the normal range in all cases.

Out of 20 SLE-sera with reduced opsonisation, 17 had increased concentrations of immune complexes (IC), detected by C1qBA in 15 cases and by semiquantitative cryoprecipitation in 2 cases. This frequency contrasted with the corresponding figure of increased immune complex concentrations in 10 of 21 SLE sera with normal opsonisation ($p < 0.05$). Antibodies to native DNA occurred in 13 of the sera with reduced opsonisation and in 10 of the sera with normal opsonisation (n.s., unpublished observation).

The opsonic capacities of 9 SLE-sera, from which cryoglobulins had been eliminated, were compared with those of the 9 corresponding, untreated SLE-sera. When the cryoglobulin fraction of IC was eliminated, opsonic capacity was restored to normal in the five cases with previous reductions. Bacterial opsonisation was simultaneously performed in 9 pool sera, to each of which had been added one separated SLE-cryoglobulin. When the results were compared with the opsonic capacity of native pool sera, a reduction of opsonic capacity was found when cryoglobulins were added from the five patient sera with previously reduced opsonisation. The relationships between the paired opsonic differences, the first found when cryoglobulin was eliminated from SLE sera and the second when the same cryoglobulin was redissolved in pool sera, were correlated ($r = 0.91$, $p < 0.01$). Thus, the anti-opsonic effect in SLE sera could be transferred, with the cryoglobulin fraction, to pool sera.

DISCUSSION

Most phagocytic bactericidal methods currently in use are based on the Maaloe technique (Maaloe, 1946) and studies of opsonisation have generally been performed with diluted sera requiring long opsonisation times (Verhoef et al., 1977; Leijh et al., 1979; Verbrugh et al., 1978). Since dilution might affect the two complement pathways differently (Lachmann, 1979), and might kinetically distort results of opsonisation (Tofte et al., 1980), we developed a modified assay for determining heat-labile opsonic capacity in non-diluted sera. With this assay, sources of error due to serum dilutions or to time factors are minimized. The monocyte and granulocyte performances in response to opsonisation of *S. aureus* were almost identical, in agreement with findings for the opsonisation of *S. pneumoniae* (Braconier and Odeberg, 1982). Disparities found in earlier studies (Peterson et al., 1977) were probably due to different assay techniques used.

Host defence against *S. aureus* infection primarily depends on phagocytosis and killing by leucocytes (Verhoef et al., 1981) after opsonisation, according to one hypothesis, by so called "natural antibodies" to peptidoglycan in the presence of complement (Wheat et al., 1974; Peterson et al., 1978; Peterson et al., 1983). Host defence against *S. aureus* 502 A primarily depends on activation of the classical complement pathway (Verhoef et al., 1977), in agreement with our findings of reduced opsonic capacity in sera from patients with C2 and C4 deficiencies, but normal opsonic capacity in sera from a properdin deficient patient.

We found reduced opsonisation in 11 of 14 SLE sera that had been collected during active disease or within a 4 month period preceding a flare. This proportion of reduced opsonisation in active disease was higher than that found by Jasin et

al. (1974), probably due to methodological differences. The more organ systems that were involved in our patients, the more pronounced was the opsonic reduction. A further illustration of the connection with SLE activity was our comparison of inactive and active phases in 8 individuals, that revealed significantly reduced opsonic capacity in conjunction with active disease.

In a hypocomplementaemic group of SLE patients, Jasin et al. (1974) found that 12 of 30 patients had reduced opsonic capacity correlating with infections in active SLE. This indicated an important role for complement in host defence, probably mostly in the "pre-antibody" phase as proposed by others (Sterzl, 1963; Williams and Quie, 1971). However, we found opsonic capacities to be reduced even in SLE-sera without reductions of complement concentrations, and the amounts of bacteria-associated activated C3 after opsonisation in SLE sera were comparable to those of controls. This discovery probably reflects the use of undiluted sera for opsonisation in our assay and perhaps the fact that our blood sampling was performed at 37 °C.

The possibility of a serum factor blocking either C3b (CR1) or Fc receptor contacts between bacteria and phagocyte was indicated by our results. Analogous findings were demonstrated by Shingu et al. (1981), but with pre-incubation of granulocytes instead of bacteria in SLE sera, and using a rosette assay for receptor detection. Several mammalian proteins are known to interact specifically with bacterial surface structures, and examples of such proteins are immunoglobulins, serum albumin, haptoglobulin, fibrinogen, fibronectin and complement components C1q (Myhre, 1981). Protein A, a type I immunoglobulin receptor, characterized by a restricted specificity interacting with the Fc parts of human IgG1, IgG2 and IgG4 (Kronvall and Williams, 1969), is found only in *Staphylococcus aureus* strains (Myhre, 1981). Since SLE is the prototypic immune-complex (IC) disease in man (Inman, 1982), IC might constitute an Fc receptor blocking factor bound to Protein A of *S. aureus*

and detected by our assay. Interestingly, protein A has been used to isolate and assay IC in SLE sera with results paralleling C1qBA (Hällgren and Wide, 1976; Faiferman and Koffler, 1982). The concentrations of IC, at least as measured by C1q--solid phase techniques, correlate with disease activity in SLE (Abrass et al., 1980), and with both renal and extrarenal manifestations (Sturfelt and Sjöholm, 1984).

When an IC fraction was eliminated from our SLE-sera by cryoprecipitation (thus preserving the undiluted technique), reduced opsonisation was restored to normal, thus indicating the involvement of IC in the reduced opsonic capacity of SLE sera; and the finding that reduced opsonic capacity could be transferred to normal pooled sera by means of the cryoprecipitate provides further support for a hypothesis of IC mediated reduction of opsonisation. Additional indirect evidence was the correlation between increased concentrations of IC as measured by C1qBA and reduced opsonisation and the normal opsonisation of the protein A deficient strain *S. aureus* Wood 46.

It has recently been shown that SLE cryoglobulins predominantly contain IgG, low amounts of IgM rheumatoid factor, and high amounts of DNA binding activity and C1q BA. The IC measured by C1q-BA in SLE sera decreased when cryoglobulins were removed. Cryoglobulins from RA patients contained more IgM rheumatoid factor and less DNA binding activity, and only very seldom bound C1q. The IC measured by C1q-BA in RA sera were not reduced when cryoprecipitate were removed (Erhardt et al., 1984). With the present method, 52 RA sera have been tested, including both cryoprecipitating sera and C1q-binding sera, though only two sera showed reductions of opsonic capacity (to be published). These findings indicate that qualitative differences between cryoprecipitate in RA and SLE sera probably are of importance for the effect on opsonic capacity.

In 4 of 13 SLE patients with reduced opsonisation a bacterial infection occurred within two months, contrasted with one failure to predict in 28 cases with normal opsonisation, indica-

ting the possibility of a more than tenfold increase of infection risk with reduced opsonisation. In contrast to Jasin's findings (1974), we did not observe any reduced opsonisation during infection, probably owing to a delay of one or two days from onset of infection to the time when blood was sampled in our study, which may have resulted in increases of acute phase reactants and probably of specific antibodies affecting opsonic capacity. Although our study thus suggests a promising predictive value of opsonisation, this finding is based on limited material.

Opsono-phagocytic killing of *E.coli* was studied with a conventional technique, and only one sera out of six collected within two months before the patients contracted lower urinary tract *E.coli* infections had reduced capacity to support opsono-phagocytic killing. Although, perhaps other mechanisms of first line defence in the urinary tract are more important than opsonisation for *E. coli*, another possibility is the insensitivity of the *E. coli* assay based on diluted sera, and a third possibility is that the serum-resistant strain chosen was not representative for other pathogenic variants of *E. coli*.

The experimental studies indicate reduced opsonisation of protein A bearing bacteria due to immune complexes to be of major importance in host defence against *S.aureus*, quite independent of the many other factors that have been considered as being more or less conducive to infection proneness in SLE patients.

GENERAL SUMMARY

An unselected group of SLE patients from a defined population (I) has been followed sequentially in an SLE clinic (II), and included in a controlled study of infection frequencies (III). The overall benign character of SLE found in the epidemiological group necessitated the inclusion of selected cases when studying the influence of disease activity on infection frequencies (III) and the in vitro opsonic capacities of SLE serum (IV).

In the health care districts of Lund and Örup, an annual incidence of 4.8 cases, a mortality of 1.3 cases, and a prevalence of 39 cases per 100,000 individuals, was found for SLE during the years of 1981 and 1982. The 1982 ARA classification criteria were fulfilled by 94 per cent of the patients, more males than females fulfilling internal organ criteria ($p < 0.03$).

The benign course of SLE in the unselected group was illustrated by the high age of most fatal cases (I,II), the high median age in the group (I), the infrequent need for hospitalisation (I,II), the low overall level of corticosteroid treatment (II), the normal employment rate (II), and the low rate of spontaneous abortion (I).

Eighteen major flares, occurring predominantly during summer ($p < 0.05$), and 13 minor flares were seen in 29 patients. A change in subset between flares was common and ESR, S-CRP, S-orosomukoid and $\bar{C}1r$ - $\bar{C}1s$ - $\bar{C}1$ IA complexes, indicating C1 activation, were increased ($p < 0.01$) in active disease.

Bacterial infections were more common among unselected SLE patients than among age and sex matched controls ($p < 0.01$), but barely significantly increased compared with age, sex and therapy matched RA patients ($p < 0.06$). Infections caused by *S. aureus* were only found in the SLE group.

With the low dose regimen of corticosteroids employed, no correlation between infections, steroid treatment or steroid dosage could be observed, though there was a tendency towards more opportunistic infections with increasing corticosteroid dosage. However, earlier prolonged corticosteroid therapy was more common among 10 SLE patients with repeated bacterial infections than among 50 SLE patients without bacterial infections ($p < 0.05$).

Bacterial infections were more common during SLE activity than during quiescent disease in 22 untreated SLE patients ($p < 0.04$), and this difference was more pronounced in 41 patients on corticosteroids ($p < 0.001$). In 49 patients, bacterial infections were more common after flare than before ($p < 0.03$). No diagnostic significance of ESR, S-CRP or leucocyte counts was found in the bacterially infected patients. Of a total of seven deaths, infections caused or contributed to five.

The high frequency of *S. aureus* infections observed clinically (III), provided a basis for the experimental work on opsonisation of *S. aureus* in SLE sera. An in vitro method allowing opsonisation in undiluted sera was developed. Heat-labile opsonins were clearly shown to be one determining factor of opsonic capacity as studied with the present assay.

Reduced opsonic capacity was more common during lupus activity than during quiescent disease ($p < 0.004$), and the degree of reduction correlated with the number of active organ systems ($p < 0.001$). Bacterial infections were more common in the two months following a reduction in opsonic capacity ($p < 0.05$). Granulocyte and monocyte killing of *S. aureus* opsonised in the same SLE sera were correlated ($p < 0.001$).

Opsonic capacity correlated neither with immunochemically determined concentrations of complement components, nor with functional complement assays or concentrations of bacteria associated activated C3. Comparisons between opsonic capacity

and corticosteroid treatment showed no consistent patterns.

When the cryoglobulin fraction of immune complexes (IC) was eliminated, normal opsonic capacity was restored, and the opsonic reduction could be transferred with the cryoglobulins to pooled serum ($r=0.91$, $p<0.01$). Increased IC values were found in conjunction with reduced opsonic capacity ($p<0.05$) and since opsonization of a protein A deficient strain of *S. aureus* was normal, reduced phagocytosis of *S.aureus* in SLE sera perhaps may be explained by IC binding to staphylococcal protein A.

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APPENDIX (I-IV)

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SYSTEMIC LUPUS ERYTHEMATOSUS IN AN ADULT POPULATION

IN SOUTHERN SWEDEN

Incidence, Prevalence and Validity

of ARA Revised Classification Criteria.

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ABSTRACT:

In a defined Swedish population an annual incidence of 4.8 cases, a mortality of 1.3 cases and a prevalence of 39 cases per 100.000 individuals was found for Systemic Lupus Erythematosus (SLE). Sensitivities were comparable (94% cf 92%) for ARA criteria of 1982 and those of 1971. Males had more internal organ criteria and showed a tendency towards having less cutaneous criteria than females. Spontaneous abortion was rare and hospitalization infrequent among the 65 patients studied.

INTRODUCTION

A gradual shift in the clinical appearance of SLE has occurred in recent decades, with an increasing proportion of mild cases (1). The preliminary ARA criteria published in 1971 (2) may have contributed to a more complete identification of benign forms of SLE. However the criteria have only rarely been applied to epidemiological studies (3,4,5), and no such work has been reported from Europe.

In the early sixties, Leonhardt (6) studied the frequency of SLE in Scania in southern Sweden, a province populated almost exclusively by caucasians, and where both internal and external migration rates are low. These circumstances, in combination with the accessibility of hospital records and reliable population statistics, provided an ideal background against which to study changes in the epidemiology of SLE. For the years 1981 and 1982 we were able to test both the preliminary and revised ARA criteria, and for the first time to assess initial symptoms in a defined population. A tenfold increase in prevalence was found as compared with Leonhardt's report, and a near normal incidence of spontaneous abortion.

PATIENTS AND METHODS

POPULATION: The total adult population on 31 december 1982 was 156.924 persons. Relevant demographic data of the community under study (the health care districts of Lund and Örup) are shown in Table I. Only adult patients over the age of 14 years were included and the same age-group constituted the population at risk.

TABLE I

POPULATION IN THE COMMUNITY STUDIED

	<u>December 31st 1982</u>		<u>Mean 1981 - 1982</u>	
Age	Male	Female	Male	Female
0-14	21 731	20 635	22 051	20 892
15-44	45 882	45 520	45 327	44 983
45-64	19 212	19 157	18 911	18 917
64<	11 571	15 582	11 503	15 452
Total	98 396	100 894	97 792	100 245

PATIENT RETRIEVAL: The University Hospital of Lund is the only hospital serving the Lund and Örup districts, and only patients referred through Lund are eligible for care at other hospitals. The social security numbers of all patients with a diagnosis of SLE during the years 1973 - 1982 at the University Hospital or in primary care clinics were extracted from computerized diagnosis registers.

In addition, where diagnosis registers were incomplete for the period under study, the outpatients files of the departments concerned, in both primary care and private clinics were screened for possible cases. All pertinent information, such as time of diagnosis, organ symptoms and classification criteria fulfilled (2,7), was then extracted from the assembled records. The ANA test was substituted for LE-cells in the 1971 set of criteria. Antibodies to native DNA was determined according to Aarden et.al.(8) and precipitating antibodies to Sm antigen was determined by double immuno-diffusion (9).

A clinical diagnosis of SLE was endorsed only if typical multisystem disease was present and features incompatible with SLE were absent. The clinical features of the patients during the present observation time are described separately, and related to disease activity and laboratory parameters (10).

Six patients whose syndromes were incomplete and did not justify a clinical diagnosis of SLE were excluded, as was one patient with psoriatic arthritis combined with myelofibrosis and seven patients with erosive, seropositive Rheumatoid Arthritis, where the presence of a Sjögren's syndrome had led to an erroneous diagnosis of SLE.

All patients with a definite clinical diagnosis of SLE, and alive on 31 December 1982 were accepted for prevalen-

ce calculation; and all cases where a definite diagnosis of SLE was made during 1981 or 1982 were accepted for incidence calculation. Sixtyfive individuals, 36 of which came from outpatient clinics, met the requirements of a clinical diagnosis of SLE and constitute the study population.

STATISTICS:Chi-square test and binomial test were used when appropriate.

RESULTS

A total of fifteen new cases were identified during the two years 1981 and 1982. The incidence was calculated per 100.000 population at risk per year in three different ways. Firstly, based on clinical SLE diagnosis; secondly, based on 4 or more ARA criteria of 1971; and thirdly, based on 4 or more ARA criteria of 1982. Age specific incidence was calculated on clinically diagnosed cases (Table II).

TABLE II

INCIDENCE OF SLE IN THE COMMUNITY DURING 1981-82.

	FEMALE		MALE		TOTAL	
	n	rate*	n	rate*	n	rate*
CLINICAL DIAGNOS						
Age 15-44	4	4.5	2	2.2	6	3.3
45-64	6	15.9	0	-	6	7.9
>64	2	6.5	1	4.4	3	5.6
Total	12	7.6	3	2.0	15	4.8
ARA 1971	11	6.9	2	1.3	13	4.2
ARA 1982	12	7.6	2	1.3	14	4.5

* Average annual number of cases per 100.000 population at risk.

Of sixtyfive patients located with a definite diagnosis of SLE and alive during the years 1981 and 1982, four died during the observation period, leaving 61 for prevalence analysis. Prevalence based on clinical diagnosis was 39 cases (30-48, 95 % confidence interval) per 100.000 population at risk. Prevalence was calculated in the same manner as incidence (cf foregoing), and the results are presented in Table III.

TABLE III

PREVALENCE OF SLE IN THE COMMUNITY ON 31 DECEMBER 1982

		FEMALE		MALE		TOTAL	
		n	rate*	n	rate*	n	rate*
CLINICAL DIAGNOSIS							
Age	15-44	27	59.3	6	13.1	33	36.1
	45-64	19	99.2	2	10.4	21	54.7
	>64	6	38.5	1	8.6	7	25.8
Total		52	64.8	9	11.7	61	38.9
ARA 1971		48	59.8	8	10.4	56	35.7
ARA 1982		50	62.3	7	9.1	57	36.3

* Cases per 100.000 population at risk.

The sensitivity of ARA criteria was tested on the present patient population, 60 of 65 patients having four or more of the 1971 criteria and 61 having four or more of the 1982 criteria. The sensitivity of the 1971 criteria was thus 92, and of the 1982 criteria 94 percent. Two clinically diagnosed patients failed to fulfill four 1971 criteria and four 1982 criteria. One was a 53 year old woman with photosensitivity, non-destructive, palindromic polyarthritis and ANA positivity, who in 1979 developed a vasospastic phenomenon in her fingers on exposure to cold, with numbness and pallor but without the classical two-phase reaction of Raynaud's phenomenon; up to now there have been no additional symptoms. The Waaler-Rose test was negative. The other case, a previously healthy 68 year old man was first seen in 1982 with dyspnoea, exudative pericarditis and positive ANA. Granular cellular casts and haematuria were present and renal biopsy showed an immune-complex nephritis. A second episode of pleuritis and lamellar atelectasis occurred in february 1983.

The median number of criteria per patient was 5 both for 1971 and 1982 criteria (Fig.1). Sex distribution in the prevalence group was 85.3 % female (n=52) and 14.7% male (n=9). The total female : male prevalence ratio was 5.8 : 1, the corresponding ratio in the age interval 15 - 44 years being 4.3 : 1. Median age was 44 years (range 18-79 years).

Initial manifestations are listed in Table IV. The median interval between initial manifestations and diagnosis was 3.0 years (range 0 - 60 years). The median age at time of diagnosis was 39 years for the whole group (range 12 - 75 years) , 40 years for females (range 12 - 75 years), and 32 years for males (range 15 - 68 years). SLE was diagnosed before 1971 in eleven cases, in nine of which diagnosis was made within one year of the initial manifestations. In the remaining 54 cases , diagnosed in 1971 or later, 17 were diagnosed within a year of the appearance of symptoms, and 17 more than 10 years after the first

Fig 1

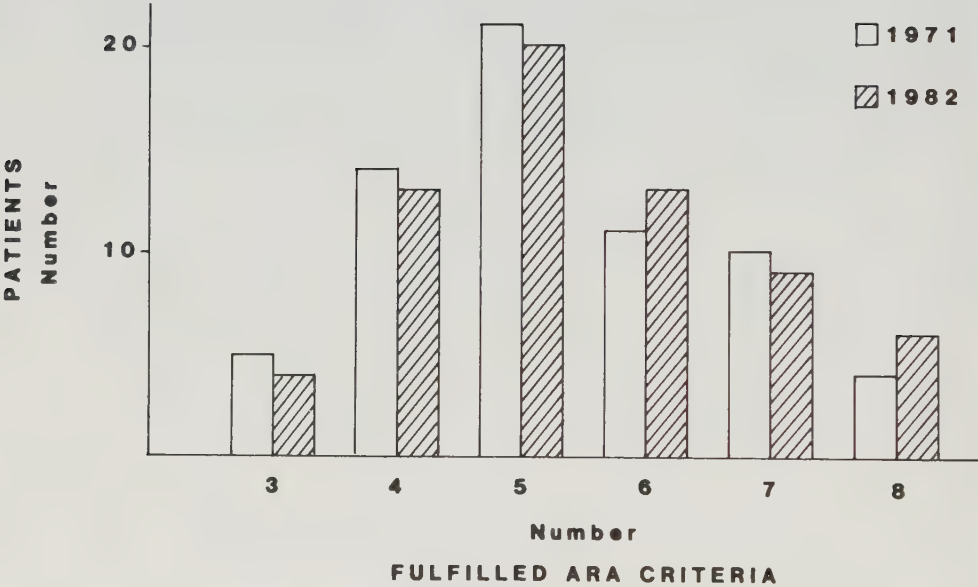


FIGURE 1: Numerical distribution of 1971 and 1982 ARA criteria fulfilled by 65 SLE patients.

TABLE IV
INITIAL MANIFESTATIONS OF SLE (n=65)

SYSTEM	n	%	MANIFESTATION	n
Musculo-articular	29	44.6	Arthritis	24
			Arthralgia	5
Cutaneous	17	26.2	Discoid lupus	6
			Photosensitivity	6
			Facial erythema	3
			Urticaria	2
Serous membranes	7	10.8	Pleurisy	5
			Dyspnoea	2
Vascular	4	6.2	Raynaud's phenomenon	4
Renal	3	4.6	Peripheral oedema (nephros)	3
Fever	2	3.1		
Neuro-psychiatric	2	3.1	Psychosis	1
			Diplopia	1
Haematological	1	1.5	Purpura	1
TOTAL	65	100.0		65

symptoms. The median duration of SLE from the time of diagnosis to the prevalence testing time was 5 years (range 0 - 32 years).

The criteria fulfilled by patients are listed in Tables V and VI. Antibodies to native DNA were found in 51% of the patients. The frequencies of patients fulfilling at least one of the 1982 muco-cutaneous criteria (Nos. 1 to 4) were 41/52 for females, and 4/9 for males ($p < 0.09$, Chi-square = 3.08). The frequencies of patients fulfilling at least one of the 1982 internal organ criteria (Nos. 6 to 8) were 28/52 for females and 9/9 for males ($p < 0.03$). The remaining criteria showed no tendency towards sex-difference.

Fortytwo of the 65 patients had been in-patients at some time during the years 1973 - 1982, and 28/65 were hospitalized for a total of 1277 days during 1981 and 1982. The median duration of each hospital admission was 8 days.

Sjögren's syndrome was present clinically and verified by lip biopsy or Rose Bengal eye-staining among 10 of the female patients (17.9%), a subgroup having a median age of 56 years (range 46-71 years). Among these patients, the median number of 1971 and 1982 ARA criteria was 5, and an increased frequency of Raynaud's phenomenon (60 %) and haematological disorder (ARA 1971 60% and ARA 1982 80%) was found.

Of the 55 female patients, 40 had been pregnant at some time, accounting for a total of 91 pregnancies, of which 82 (=90%) resulted in a live birth and 9 pregnancies (=10%) in six women ended with spontaneous abortion. Of these six women one had nephritis, two leucopenia, one of which with false positive Wasserman and the other with increased fibrinolysis, while the three remaining patients had mild and inactive SLE without any special markers.

TABLE V
1971 ARA CRITERIA FULFILLED (percent) , n=65

CRITERION	This study ARA 1971 Fessel		
Facial erythema	43	64	19
Discoid lupus	23	17	38
Raynaud's phenomenon	40	20	16
Alopecia	9	43	33
Photosensitivity	54	37	27
Oral or nasopharyngeal ulceration	14	15	8
Arthritis without deformity	97	86	95
ANA (LE-cells)	100	(92)	47
Wasserman (Chronic false positive STS)	6	12	13
Profuse proteinuria	6	16	3
Cellular casts	28	16	11
Serositis	54	60	34
Neurological disorder	9	20	13
Haematological disorder	44	52	55

TABLE VI

1982 ARA CRITERIA FULFILLED (percent) , n=65

CRITERION	This study	ARA 1982
Malar rash	43	57
Discoid rash	23	18
Photosensitivity	54	43
Oral ulcers	14	27
Arthritis	97	86
Serositis	54	56
Renal disorder	34	51
Neurological disorder	9	20
Haematological disorder	59	59
Immunological disorder	57	85
Antinuclear antibody	100	99

Four patients with a clinical SLE diagnosis, and satisfying at least 4 of the 1971 ARA criteria and at least 4 of the 1982 ARA criteria, died during 1981 or 1982. They were all women aged 79, 77, 75 and 63 years with an SLE-duration of 9, 2, 23 and 30 years respectively. The deaths were caused by myocardial infarction complicated by bronchopneumonia, oral carcinoma, septicaemia and cerebrovascular disease with bronchopneumonia. The mortality rate was 1.3/100.000 population/year.

DISCUSSION

The present work is the first epidemiological study of SLE in a Scandinavian population since the adoption of criteria for classification in 1971. Only three earlier epidemiological studies, one from San Francisco (3), one of North American Indians (4) and one from New Zealand (5), but none from Europe, have been based on defined populations with patient retrieval from both out-patient clinics and hospital admissions. The defined population was homogeneous and probably included all in-patients and out-patients with an SLE-diagnosis in the community. The completeness of the patient list was thoroughly cross-checked with the aid of separate computer-registers and personal communications with physicians practising in the actual area and in two nearby centers.

That thirtyseven of the 65 patients included in the study did not require hospital admission during the years of 1981 and 1982 is an indication of the benign character of SLE. A good prognosis is suggested by the high median age and the deaths occurring after 60 years of age. The introduction of classification criteria (2) in combination with increasing knowledge of SLE and an expanding number of qualified doctors in Sweden have contributed to the identification of a larger proportion of mild cases of SLE. The long duration between initial manifestations and the diagnosis of SLE in about one third of the patients diagnosed since 1971 might be a consequence of recent identification of mild SLE, that previously had escaped detection or been diagnosed as something else. That the incidence calculated in this study exceeds the mortality rate, is a further indication that we are dealing with an expanding diagnostic group.

The finding among the men in our study to fulfill more

internal organ criteria, and the tendency towards fulfilling less mucocutaneous criteria, than the females, may reflect different clinical pictures in the sexes. If so, SLE in males might remain clinically unidentified longer if doctors are looking for symptoms that in fact chiefly belong to the female clinical picture. The female preponderance first described by Baehr in 1935 (11) and confirmed by all later reports, is once again verified in our study. In contrast to earlier reports (12,13), however, we found a somewhat higher male proportion among younger individuals, though the numbers involved are too small for any definite conclusions to be drawn.

The SLE incidence of 4.2/100.000 found in the present study is somewhat lower than the value of 7.6 found by Fessel(3), a possible explanation for this discrepancy being that 30 % of the patients in San Francisco were black. In Table VII our prevalence rates are compared with calculated, age-adjusted prevalences from Fessel's (3) and Medding's (5) studies. The total adult prevalence of SLE in southern Sweden 1982 seems to be somewhat lower than the corresponding value found in the city and county of San Francisco in 1973, the small difference possibly being partly explainable by the heterogeneous non-black population of San Francisco. The overall impression, however, is that of good agreement in SLE prevalences between these two parts of the world. The prevalence found in a chiefly white New Zealand population in 1976 was less than half of that in this series (5), a discrepancy for which we are unable to offer any explanation.

Leonhardt studied incidence and prevalence in Scania and the city of Malmö in southern Sweden in the early sixties (6), since our community is a part of Scania outside Malmö comparison is of interest. The annual incidence of definite SLE between 1956 and 1961 was 1.0/100.000 in Malmö and 0.5/100.000 in the rest of Scania. The prevalences in 1961 were 1/16.667 for Malmö and 1/33.333 for the rest of Scania. Although the possibility that SLE-

TABLE VII

PREVALENCE OF SLE

(> 14 years of age)

Cases with 4 or more 1971 ARA criteria fulfilled

Cases per 100.000 population at risk

STUDY		FEMALE	MALE	TOTAL
SOUTHERN SWEDEN 1982				
(Present)	Total	59.8	10.4	35.7
SAN FRANCISCO 1973				
(Fessel)	Total	116.0	14.3	65.2
	Non-black	91.7	9.0	50.3
	Black	362.3	67.8	214.6
DUNEDIN 1976				
(Medding)	Total	30.9	7.6	19.7

frequency is increasing cannot be ruled out, greater awareness of the syndrome, and the introduction of the SLE criteria probably account for much of the tenfold increase. Interestingly, the SLE incidence and prevalence of Leonhardt's study was compared with those of New York City in the early sixties (13), and a good correlation was found.

A rather close similarity is apparent between the initial manifestations in this study and those reported by Estes and Christian (14), the main difference being a higher proportion of pleurisy and pericarditis in the present study, an observation that needs confirming in larger series.

The frequencies of ARA criteria fulfilled do not seem to have been reported on an epidemiological basis before but rather on large numbers of referred or selected patient groups (i.e. 1,15). When comparing our patients with the frequencies published together with the 1971 ARA criteria (2, Table V) and with the 1982 ARA criteria (7, Table VI) most frequencies are quite in agreement. Exceptions being those for renal and neurological disorders which are less common in our material. These discrepancies most probably reflect a higher proportion of cases with more complete SLE in the group studied to establish criteria than in our study. When testing the ARA criteria for classification of SLE in our clinical population, a good sensitivity of 92 % was arrived at with the old criteria and 94% with the revised criteria. This is in agreement with earlier sensitivity studies of the 1971 criteria (16).

The clinical frequency of Sjögren's syndrome in our study (17.9%) is comparable to that usually reported (17), but lower than the 46 % reported in one study by Alarcon - Segovia (18), differences possibly due to different diagnostic procedures.

Spontaneous abortion has been reported to be more common

in pregnancies with SLE mothers, with frequencies as high as 25 - 46 % (19). In our epidemiological study the abortion frequency in 40 women of 9/91 pregnancies was significantly lower ($p < 0.001$) than the corresponding value in 39 women (50/137) reported by Grigor (19) and comparable to the 10 % reported in pregnancies among the general swedish female population (20). Since, however, the latter figure includes an estimated frequency of missed abortion, the epidemiological SLE group may nonetheless represent a slight indeterminate increase of spontaneous abortions.

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Submitted for publication

CLINICAL INCONSISTENCY, BENIGN COURSE AND NORMAL EMPLOYMENT
RATES IN UNSELECTED SYSTEMIC LUPUS ERYTHEMATOSUS

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SUMMARY

Of 65 unselected SLE patients from a defined population 61 were sequentially followed for two years with an SLE supervision programme. The cumulative clinical manifestations included a relatively high frequency of pulmonary vascular disease and gastrointestinal involvement. Flares were more common during the summer season and a change in the clinical manifestations occurred in 10 of 16 patients with major flares and in 4 of 13 patients with minor flares. ESR, S-orosomucoid, S-CRP, and $\text{C}\bar{\text{I}}\text{r}-\text{C}\bar{\text{I}}\text{s}-\text{C}\bar{\text{I}}$ IA complexes, indicating C1 activation, were shown to distinguish inactive from active disease. The prevailing overall benignancy was reflected in a low mortality rate, limited need for treatment or hospitalisation, and good response to moderate dosages of corticosteroids in severe flares. The proportion of patients in gainful employment was comparable to that in the normal population, though absence due to sickness was more common in the SLE group. Joint complaints and mild psychiatric disturbance were the most common causes of enduring incapacity.

INTRODUCTION

The modern concept of SLE is that of a benign syndrome, and its clinical spectrum is very broad and heterogeneous. Much of the clinical and immunological information on SLE derives from studies of selected materials and needs to be confirmed by studies on unselected groups of patients. Few prospective studies of SLE have been performed on defined populations.

In 1980, an SLE clinic at the Department of Rheumatology in Lund was started as a centre for the supervision of SLE patients in the province of Scania in southern Sweden. The present paper is based on a two year observation period of all SLE patients identified within a defined population.

MATERIALS AND METHODS

The SLE clinic in Lund

In 1980 we organized an SLE clinic staffed by two physicians, a nurse, laboratory assistant and a secretary. Its purpose was the primary care of all SLE patients within the health care-districts of Lund and Örup, and to provide a consulting service for patients from southern Sweden.

Patients with a clinical diagnosis of SLE (see below), seen at the SLE clinic, were consecutively included in a standardised supervision programme. By the 1st of January 1984, 150 SLE patients had been seen by the group and 95 patients were being followed sequentially at intervals of 6 to 8 weeks, or as appropriate to the course of the disease. Standardised clinical examinations and routine laboratory investigations were carried out on each occasion, and serum and EDTA-plasma were col-

lected.

When appropriate with regard to organ involvement, clinical evaluation and medical treatment of patients were arranged in consultation with clinicians at the Departments of Infectious diseases, Neurology, Nephrology, Psychiatry, and Dermatology at the University Hospital in Lund.

Diagnostic principles

A diagnosis of SLE was based on a series of features of multi-system disease, particularly of skin, joints, serosal membranes, kidneys, lungs, nervous system and blood (1). Diagnosis was also supported by unexplained fever, adverse reactions to drugs, multiple abortion and thrombo-embolic disease. Monosystem disease was thus excluded.

A diagnosis of SLE was supported by the presence of one or several immunological abnormalities, such as the occurrence of antinuclear antibodies (ANA), anti-native DNA (nDNA) antibodies, anti-Sm antibodies, evidence of genetic deficiency of complement or activation of the complement classical pathway. However, neither ANA positivity (2), nor the presence of anti-nDNA antibodies was a sine qua non for a diagnosis of SLE.

Patients were excluded from the study if they had any other distinct systemic connective tissue disorders, such as rheumatoid arthritis, progressive systemic sclerosis, dermatomyositis, polyarteritis, or any syndrome resembling SLE, such as chronic cutaneous vasculitis, drug induced lupus syndromes or primary Sjögren's syndrome. In cases of differential diagnostic difficulty (e.g., overlap cases), a diagnosis of SLE was favoured by the presence of butterfly rash, glomerular disease verified by biopsy, anti-nDNA antibodies, anti Sm-antibodies, absence of positive Waaler-Rose test or absence of erosive arthritis. Clinical diagnosis was considered to be established only when unreservedly endorsed by all doctors involved in the

case under consideration. The ARA criteria of 1971 (3) and 1982 (4) were not used in diagnosis, but solely for classification purposes. Diagnosis was continuously re-evaluated at each attendance of the patients at the clinic.

Patients

The population under study was composed of adults living within the health care districts of Lund and Örup (demographic data and details of the patient retrieval system are presented elsewhere,(5)).

Sixtyone patients, 52 females and 9 males, median age 44 years, were included in the prospective study and followed. Median disease duration from diagnosis was four years (range 0-32 years). From the defined population, a further four patients were not included in the sequential study - two who were seen only once during the study period, and two who had been seen earlier but who refused to attend the SLE clinic. In fifteen, of the total 65 patients, SLE was diagnosed during the study period. Four or more of the ARA classification criteria for SLE (4) were fulfilled for all but four of the total of 65 patients. In accordance with the diagnostic principles outlined above, 37 patients with presumptive SLE diagnosis were excluded - 14 with a previous diagnosis of SLE and 23 with other systemic connective tissue disorders.

Laboratory investigations

ESR, S-Hb, leucocytes, platelets, liver enzymes, S-creatinine, S-orosomucoid, and S-CRP were determined by routine procedures. Complement components C1q, C4, C3, and factor D were determined by electroimmunoassay (6,7). Complexes containing C $\bar{\text{T}}\text{r}$, C $\bar{\text{T}}\text{s}$, and C $\bar{\text{T}}$ inactivator (C $\bar{\text{T}}\text{r}$ -C $\bar{\text{T}}\text{s}$ C $\bar{\text{T}}$ IA) and C3d were determined by electroimmunoassay as previously described (8,9). Circulating immune complexes were measured by a C1q solid phase assay (10). Antinuclear antibodies were determined by indirect immunofluorescence and precipitating antibodies to

nuclear ribonucleoprotein (RNP) and to Sm antigen by immunodiffusion (11). Anti-nDNA antibodies were determined by the Crithidia Luciliae test (12). Rheumatoid factors were measured by Waaler-Rose test (13). Serological tests were kindly performed at Department of Immunology by Dr A.G. Sjöholm.

Blood sampling.

Routine laboratory tests were carried out at each visit of a patient to the clinic. For further immunological tests, blood was sampled at all clinical attendances, and venesection was performed at scheduled times. Clotting was allowed to proceed for one hour at room temperature ($+22^{\circ}$ C) followed by one hour at $+4^{\circ}$ C, blood collected in EDTA at 5 mmol/l being treated in the same way. After centrifugation, EDTA-plasma and serum were frozen in aliquots and stored at -80° C.

Evaluation of clinical disease activity

Features of active disease during the study were divided into minor and major manifestations as previously described (14). Briefly, minor manifestations (usually arthritis in combination with skin manifestations) could safely be treated with antimalarial drugs, low dosages of Prednisolone (<20 mg/day), or both. Major manifestations, on the other hand, always warranted hospitalisation and treatment with Prednisolone at dosages of more than 20 mg/day, sometimes in combination with other immunosuppressive drugs.

Renal disease was considered active if one or more of the following observations were made:

1. development of haematuria, pathological casts, or both.
2. impairment of renal function (CrEDTA clearance).
3. onset of proteinuria (>0.5 g/24 hours).

Neuropsychiatric manifestations were clinically evaluated and assessed by laboratory investigation, including computerized tomography, visual response EEG, cerebral blood flow determi-

nations (Xenon₁₃₃), and routine liquor analysis.

Serositis (pleuritis, pericarditis) was diagnosed by pain accompanied by a rub, an effusion, abnormal chest x-ray or ECG changes consistent with pericarditis. Lung vasculature was evaluated by ventilation perfusion scintigraphy (15). In all patients with fever or major manifestations, investigation included cultures from blood, urine and nasopharynx and serological screening for viral infections, additional investigations being performed as appropriate to the clinical picture.

Major infections

Major clinical infections, with such systemic manifestations as fever or malaise and requiring systemic antibiotic treatment, were confirmed by positive cultures, and by radiographic evidence or prompt regress in response to antibiotic therapy (16).

Statistics

Differences between values obtained at active and at inactive disease were evaluated by the Wilcoxon signed rank test for matched pairs (17).

RESULTS

Clinical features of the study group

Cumulative clinical manifestations of the study group are summarised in Table I. Most patients showed signs of inflammatory joint disease, and in three such patients erosive changes were identified by X-ray; all of them were anti-n DNA positive and Waaler-Rose negative, and one was a 44 year old woman who also had proliferative glomerulonephritis, pleuritis, leucopenia, lymphadenopathy and cytooid bodies. One patient was a 37 year old woman whose disease had started as a chronic juvenile arthritis with Sjögren's syndrome; later she developed pleuritis, pericarditis, sun sensitivity and proliferative glomerulonephritis. Another patient was a 47 year old woman presenting with a butterfly rash, arthritis, ulcerous colitis and Sjögren's syndrome. This patient had multiple erosive lesions with deformity of the wrists and interphalangeal joints.

The main mucocutaneous manifestations in the patients are given in Table II.

Forty-one patients (63 %) had histories of cardiopulmonary manifestations. The most common lesion was pleuro-pericarditis, diagnosed in 35 patients (54 %). Pulmonary vascular disease, with ventilation-perfusion disturbances had been found in 9 patients (14 %). Myocardial infarction had occurred in 4 women, with premenopausal development at the age of 36 in one case, and postmenopausal development of cardiac ischaemic lesions in the other three. Five patients had histories of myocarditis without pericardial effusion, two of mitral valve disease, and one of restrictive cardiomyopathy.

Table I

CUMULATIVE CLINICAL MANIFESTATIONS IN 65 SLE PATIENTS

<u>Disorder</u>	<u>Number of patients (%)</u>
Arthritis, arthralgia	64 (97 %)
Mucocutaneous lesions	50 (77 %)
Cardio-pulmonary manifestations	41 (63 %)
Peripheral vascular disease	33 (51 %)
Nephritis	17 (26 %)
Neuropsychiatric manifestations	16 (25 %)
Gastro-intestinal manifestations	11 (17 %)

Table II

n = 65

MAIN CUMULATIVE MUCOCUTANEOUS MANIFESTATIONS
IN THE SLE PATIENTS

<u>Disorder</u>	<u>Number of patients (%)</u>
Butterfly erythema	28 (43 %)
Severe phototoxic reactions	24 (37 %)
Discoid lesions	15 (23 %)
Alopecia	6 (9 %)
Severe oral ulcers	4 (6 %)
Widespread exanthema	2 (3 %)
Telangiectasia	2 (3 %)
Vitiligo	2 (3 %)

Renal involvement justifying a diagnosis of nephritis had been observed in 17 patients, of whom some clinical features and results of renal biopsies are given in Tables IIIa and IIIb. Immunofluorescence revealed glomerular deposits of immunoglobulins and complement components in all biopsy-specimens.

Table IIIa
CUMULATIVE CLINICAL FEATURES OF NEPHRITIS
IN 17 SLE PATIENTS

Disorder		Number of patients
Proteinuria	>2 g/24 h	9
	0.5-2 g/24 h	5
	<0.5 g/24 h	3
Haematuria		10
Cylindriuria		16
Renal function	GFR <20 ml/min/1.72 m ²	3
	20-60 ml/min/1.72 m ²	3
	>60 ml/min/1.72 m ²	11

Table III b

RESULTS OF RENAL BIOPSIES IN 17 SLE PATIENTS WITH NEPHRITIS

Morphological picture	Number of patients
Proliferative glomerulonephritis	4
Membrano-proliferative glomerulonephritis	2
Epimembranous glomerulonephritis	1
Unclassifiable glomerulonephritis	4
Renal biopsy not performed	6

Table IV

CUMULATIVE NEUROPSYCHIATRIC MANIFESTATIONS

n = 65

Disorder	Number of patients (%)
Depressive illness	7 (11 %)
Cranial nerve lesions	4 (6 %)
Chronic brain syndrome	2 (3 %)
Myelitis	2 (3 %)
Epilepsia	1 (1 %)
Polyradiculitis	1 (1 %)

Of sixteen patients with histories of neuropsychiatric SLE (Table IV), only one had seizures.

In 11 patients (18 %) severe, SLE associated, gastro-intestinal manifestations had been present during the course of the disease. Inflammatory hepatopathy, unconnected with medication, was diagnosed in seven patients, in whom no evidence was found of any specific liver disorder, such as primary biliary cirrhosis or chronic active hepatitis, and liver biopsies showed unspecific reactive hepatitis. Of the 11 patients with gastro-intestinal manifestations, severe abdominal pain during clinical flare was found to be due to pancreatitis in two cases, and to aseptic peritonitis in one case. One patient had multiple mucosal ulcerations in the esophagus, stomach, duodenum, and colon; since microscopic evaluation of biopsies showed no evidence of vasculitis, the lesions were considered to have been induced by analgetic drugs. As already mentioned, another patient had ulcerous colitis.

Table V

CUMULATIVE PERIPHERAL VASCULAR DISEASE

n = 65

Disorder	Number of patients (%)
Raynaud's phenomenon	26 (40 %)
Thrombosis, thrombophlebitis	13 (20 %)
Cutaneous vasculitic ulcers	3 (5 %)
Gangrene of extremities	2 (3 %)
Osteonecrosis	1 (2 %)

Thirty-three patients had various forms of peripheral vascular disease (Table V). No obvious relationship was seen between peripheral vascular disease and the occurrence of neuropsychiatric or renal involvement.

Sjögren's syndrome (18) and symptoms of keratoconjunctivitis were present in 13 patients, all women, of whom eight had stainable (Rose Bengal) lesions, six had xerostomia as well, six had thyroid disease, and two had renal calculi. Multiple drug reactions, seen in seven of the 65 patients, were not more common among patients with Sjögren's syndrome.

Only two women in the group studied had histories of multiple abortion.

Three out of five males had no history of skin or mucous membrane symptoms, as compared with only 12 out of 56 women, though this difference was not significant. In five out of nine male patients, retrospective analysis showed that the onset of SLE had been acute, involving several internal organ systems including the kidneys; all five cases responded readily to corticosteroid treatment and no further flares occurred over a mean observation period of eight years.

Table VI
ANTINUCLEAR ANTIBODIES AND RHEUMATOID FACTORS
IN 65 SLE PATIENTS

<u>Test</u>	<u>Per cent positive patients</u>
Antinuclear antibodies (ANA) (indirect immunofluorescence)	100
Anti-nDNA (Chrithidia luciliae)	59
ENA	28
anti-RNP	22
anti-Sm	6
anti-SS-B	4
Waeler-Rose	6

The serological profile

Data on ANA, anti-nDNA antibodies, anti-Sm antibodies and Waeler-Rose titres are given in Table VI. Out of 17 patients with renal involvement 15 were anti-nDNA positive. Antibodies against soluble antigens tested were not associated with Raynaud's phenomenon or any other clinical manifestations. No case of homozygous complement deficiency was found. One patient had an IgA deficiency.

Clinical observations during the study period

During the study period SLE diagnosis was made in 15 patients who were thus included in the study bringing the total up to 65, of whom four subsequently died.

Of the four who died, the cause of death was probably related to SLE manifestations in one case; a 75 year old woman who died of acute myocardial infarction during a flare of polyra-

diculitis. Another woman, aged 78, died from arteriosclerotic heart disease with cardiac decompensation, one 78 year old woman from malignant palate carcinoma and a 63 year old woman from cerebrovascular disease with secondary bronchopneumonia.

Twenty-eight patients were admitted to hospital for an aggregate of 46 in-patient episodes during the observation period.

Sixteen patients had major flares, the main clinical features of which are given in Table VII. During these flares, most patients with severe SLE manifestations had fever and minor skin and joint symptoms which are not listed. The neuropsychiatric manifestations included myelitis in two patients and polyradiculitis, peripheral neuropathy and hemianopia in one patient each. Gastro-intestinal involvement was observed in two patients, one of whom presented with hepatopathy and the other with aseptic peritoneal serositis.

Minor flares were seen in 13 patients with predominantly skin and joint symptoms (Table VIII). Two patients had neuropsychiatric symptoms, epilepsy in one case and peripheral neuropathy in the other. One patient had mild myocarditis with ECG changes including conduction aberration and fatigue.

Among those whose disease had started before the study period, a change in clinical manifestations was seen in 10 patients with major flares and in four patients with minor flares, while the same clinical picture as during previous flares was seen in four patients with major flares and eight patients with minor flares.

Major flares occurred more often ($p < 0.05$) during the summer season ($n=14$) than between September and March ($n=4$). In five patients who had minor or major flares between September and March, flare was suspected of being related to previous bacterial infection in two cases, to cessation of chloroquine therapy in one case, and to exposure to ultra-violet rays in two cases.

Table VII

MAIN CLINICAL FINDINGS AT MAJOR FLARES IN 16 PATIENTS DURING
THE STUDY PERIOD

Only one flare was seen in each patient with the exception
of two patients, one with renal involvement and one with
pulmonary vasculitis both of whom had 2 flares

<u>Disorder</u>	<u>Number of patients (%)</u>
Neuropsychiatric manifestations	5 (31 %)
Pericarditis/pleuritis	5 (31 %)
Pulmonary vasculitis/embolism	4 (25 %)
Glomerulonephritis	3 (17 %)
Myocardial infarction	2 (13 %)
Raynaud's phenomenon	2 (13 %)
Gastro-intestinal involvement	2 (13 %)
Cardiomyopathy	1 (6 %)
Gangrene of extremities	1 (6 %)

Table VIII

MAIN FINDINGS AT MINOR FLARES IN 13 PATIENTS DURING THE
STUDY PERIOD

Only one flare was seen in each patient

<u>Disorder</u>	<u>Number of patients (%)</u>
Arthritis	7 (54 %)
Cutaneous manifestations	6 (46 %)
Fever	4 (31 %)
Neuropsychiatric manifestations	2 (15 %)
Raynaud's phenomenon	2 (15 %)
Myocarditis	1 (8 %)

Laboratory data at clinical flare (Table IX)

ESR, S-CRP and S-orosomucoid increased in active disease, while other routine laboratory tests showed no difference between high and low disease activity. High CRP levels (>100 mg/l) were observed in 3 patients with pleuro-pericarditis in whom no evidence of infection could be detected, all promptly responding to corticosteroid treatment. In patients with major flares C1q binding immune complexes were high in active SLE, and the difference between active and inactive values was significant ($p<0.01$). However, in patients with minor flares no such difference was found. For complement components C1q, C4 and C3 no consistent pattern was seen. Ten patients had during the study continuously low values of C4 (<53 % of the normal reference). Increased amounts of C1r-C1s-C1 IA complexes (indicating C1 activation) were, however, found in 28 of 29 patients, and the difference between values at high and low disease activity was highly significant ($p<0.01$). In the 29th patient with a minor flare and seizures, but no other evidence of active organ manifestations, C1r-C1s-C1 IA concentrations were within the normal range. Increased concentrations of C3d were found only in 9 patients during active disease. The highest concentrations were found in 5 patients with major flares. The serum concentrations of factor D, of the alternative pathway of complement, were increased in three patients with renal insufficiency.

Infections

Twenty-six major bacterial infections, with such general symptoms as fever and malaise, and requiring systemic antibiotic treatment, occurred in 18 patients. Of these infections, 12 were localised to the upper respiratory tract, 7 were pneumonia, 3 were cutaneous abscesses, 2 pyelonephritis, 1 osteomyelitis and 1 septicaemia. The pneumonias included bronchopneumonias in the 4 elderly SLE patients that died during course of the study.

Table IX

COMPARISON OF LABORATORY DATA OBTAINED AT ACTIVE AND INACTIVE
DISEASE IN 27 OF 29 SLE PATIENTS WITH FLARES DURING
THE STUDY PERIOD

Significances of differences were tested with
Wilcoxon's matched pairs signed rank test

Haemoglobin	n.s.
Leucocytes	n.s.
Lymphocytes	n.s.
Platelets	n.s.
ESR	p<0.01
S-ASAT	n.s.
S-ALAT	n.s.
S-Creatinine	n.s.
S-CRP	p<0.01
S-Orosomucoid	p<0.01
C1q	n.s.
C4	n.s.
C3	n.s.
D	n.s.
C $\bar{1}$ _r -C $\bar{1}$ _s -C $\bar{1}$ _A	p<0.01
C3d	n.s.
C1qSP	n.s.

n.s. = not significant

ASAT = Aspartat-aminotransferas

ALAT = Alanin-aminotransferas

Work incapacity

Of the 54 patients below retirement age, 43 (80%) were gainfully employed during 1982, a figure comparable to that of 83% for the total population matched with regard to age and sex (19). Seven of the 54 patients were drawing early retirement pension, and the increase in sickness absence among the remaining 47 patients, is compared with official national statistics (19), in Figure 1. Of the annual totals of days of work incapacity, 95 per cent were based on medical certification and five per cent on self-certification. According to patient records, 37 per cent of sickness leave was caused by joint related symptoms and 49 per cent by other SLE manifestations of internal organ involvement. Long term incapacity, defined as more than 90 days per annum, occurred in nine cases, in four of which joint problems predominated; in three of the remaining five cases, anxiety, debility and fatigue were important features.

Treatment

Thirty-one patients were not treated with corticosteroids at all during the study. In 32 patients treated with Prednisolone, the mean dosage during the study period was 7.5 mg/day. Seventeen patients were treated with oxy-chloroquine, and 8 with Azathioprine. Plasma exchange was performed in two patients with active renal involvement.

Obstetric and gynaecological manifestations

Five pregnancies occurred in 5 women during the study, three resulting in normal deliveries and two in spontaneous abortion. All five pregnancies occurred during complete clinical remission.

Three patients were on mixed hormone contraceptive tablets without obvious side effects or the course of their SLE being affected.

DURATION OF SICKNESS ABSENCE IN UNSELECTED SLE COMPARED WITH THE TOTAL SWEDISH POPULATION

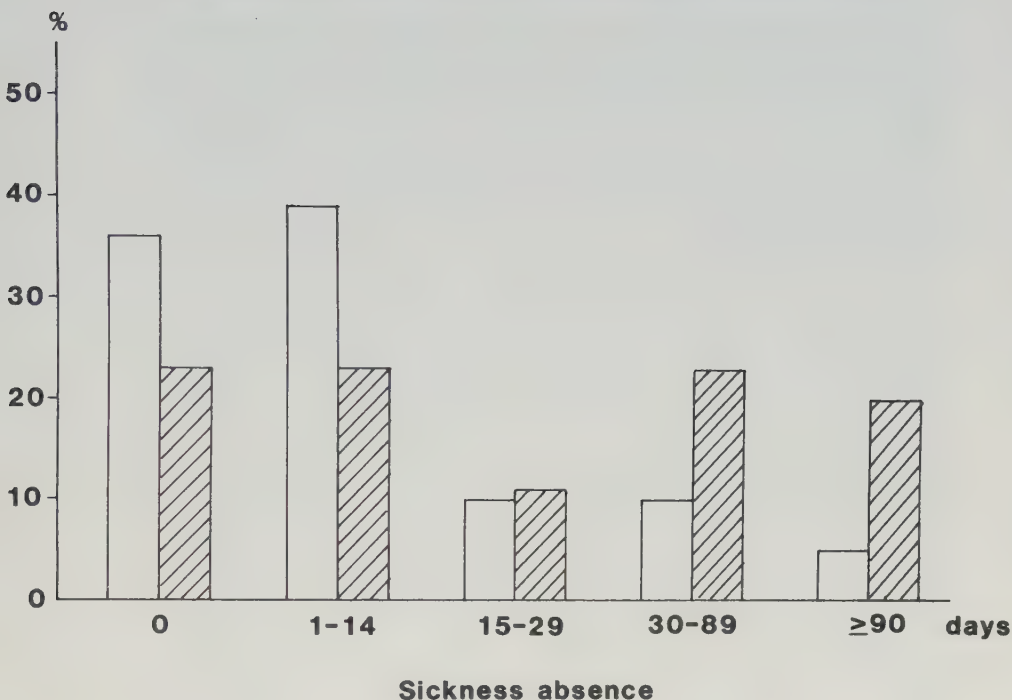


Figure 1.

Duration of stratified sickness absence during 1982 in unselected SLE ($n = 47$, median = 20 days, hatched columns) compared with the corresponding figure in total Swedish population (median = 3 days, open columns). The increase in the SLE group is significant ($p < 0.004$, combined median = 6 days, median test).

DISCUSSION

Available clinical descriptions and laboratory investigations of the SLE syndrome are almost exclusively based on studies of selected cases (20,21,22,23). Comparison between series with different selection principles from unspecified populations are, of course, hard to interpret. Epidemiological studies, on the other hand, have been available to a very limited extent from a few centres with racially mixed populations (24,25).

In the present investigation, a systematical patient retrieval from a defined adult population in a part of Scania, the southern province of Sweden, has provided the basis for an unselected patient group (5). Owing to the unique computer based health care system, even for out-patients, in this area, with only one hospital serving the population, suspected or diagnosed cases could be accurately traced. Crosschecking by personal communication and separate computer registers have verified the degree of thoroughness achieved. Since, however, additional mild undiagnosed cases might, of course, exist, the number of patients observed in the study may be regarded as a minimum for the region.

One feature arising from this study was the importance of a continuous re-evaluation of the SLE diagnosis. This was illustrated by the case of a patient who entered the study with SLE diagnosis due to a clinical picture with palindromic arthritis, Raynaud's phenomenon, pleuritis, positive ANA, positive tests for antibodies towards nuclear antigens ENA and SS-B. During course of the study the patient developed scleroderma features such as facial and thoracic telangiectasia, sclerodactyli, and dysphagia. Thus, the limits between SLE and overlap syndromes to other inflammatory connective tissue diseases are vague and transition from one entity to another is sometimes seen (26). Furthermore, this study confirms that, within the SLE population patients with destructive arthritis do occur (27), thus aggravating diagnostic difficulties, espe-

cially in patients with associated Sjögren's syndrome.

The prevailing benignancy of the SLE syndrome was reflected by the fact that less than 50 per cent of the patients needed hospitalisation during the study period. Four patients died, all at high age and only one of them due to active SLE manifestations. Most clinical flares, even those with severe manifestations of internal organ involvement, could be managed by moderate dosages of corticosteroids (≤ 40 mg/day Prednisolone). This is perhaps reflected by the comparably low incidence of severe infectious complications among our patients (16). Chloroquine therapy was used as the standard drug to maintain remission. In several cases, cessation of antimalarial therapy was followed by minor or major flare.

Another probable reflection of the benignancy of the SLE syndrome was the fact that the proportion of SLE patients in gainful employment was comparable to that found in the normal population, even if, as expected, absence due to sickness was more common in the SLE group. An important observation was that long-term incapacity to work was more often due to joint problems and to mild psychiatric disturbances than to manifestations of internal organ involvement.

The relatively high frequency of pulmonary vascular disease, in our material is noteworthy; the availability of modern diagnostic facilities probably contributed to this finding. Furthermore, it was obvious that gastro-intestinal manifestations, other than those induced by drugs, are fairly common in SLE (28). The frequency of lupus nephritis was lower in our study than in several other previous reports (20,21,22). Apart from these findings the distribution of clinical manifestations in our patients corresponded well to that to be found in other reports of large groups of selected SLE-patients (20,21,22).

A silent course after an initial dramatic flare with internal organ involvement was more common among men than among women.

This difference in the clinical picture of lupus between the sexes merit further attention. It might turn out to be related to immune modulation by sex-hormones (29).

The present findings of pronounced acute phase response during active disease, probably reflect the frequent occurrence of extrarenal inflammatory manifestations in unselected SLE. Thus, assays for complement activation appeared to provide more useful information on monitoring disease activity than did measurement of complement component levels (30). Besides the high frequency of anti-nDNA positivity in lupus glomerulonephritis, serological profiles provided little information as to clinical manifestations in the group studied.

Exacerbation of SLE manifestations was more common during the summer than during winter, probably owing to inevitable exposure to sunlight during summer. In some contrast to the findings of Fries (1), a change in clinical manifestations from one period of high disease activity to another was not uncommon. This suggests that physicians looking after SLE patients should be continually alert for possible disease activity in various organ systems.

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SYSTEMIC LUPUS ERYTHEMATOSUS AND INFECTION

A controlled and prospective study including an epidemio-
logical group.

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SUMMARY

162 infections were observed in an epidemiological group of 60 SLE patients, and prospectively followed for an aggregate of 1366 months. Bacterial infection was more frequent in the SLE-patients than in matched controls and barely more frequent than in matched RA patients. The RA patients, also matched with regard to corticosteroid dosage, showed only a tendency towards an increased infection rate. Lower urinary tract infections were comparable in all three matched groups. In a total of 102 SLE patients studied, including the epidemiological group, clinical SLE activity was accompanied by an increase in the frequency of bacterial infection. In 49 patients bacterial infections were more common after flare than before. There was a high incidence of mucocutaneous infections induced by *S.aureus*, where skin traumatization was an added risk factor. No increase of opportunistic infections was found in matched groups, but 26 such infections occurred in the subjects as a whole, and they were seen to be more frequent in conjunction with steroid treated active SLE. Admission to hospital because of suspected infection was common, and infection was the cause or a contributory factor in five of the seven deaths among SLE patients.

INTRODUCTION

An increased rate of infection among patients with Systemic Lupus Erythematosus (SLE) has long been proposed (1,2,3), and infection is one of the principal contributory factors in SLE mortality (4,5). Of several factors thought to predispose to infection in SLE that have hitherto been analyzed, only corticosteroid treatment, active urinary sediment (as a renal activity indicator), and uremia (even without evidence of active nephritis), have actually been correlated to increased frequency of infection (6,7,8). However, as was commented in a recent review of infection in immunologically-mediated diseases (9), there are still no objective clinical data to support the existence of any relationship between the SLE--syndrome and rates of infection.

The present controlled and prospective study was designed to determine the frequency and range of infections in a defined unselected group of SLE patients. Bacterial infections, especially those due to *S.aureus*, were more common in SLE patients than among matched controls. The temporal relationship between clinical SLE-activity and infection was studied in a larger group of SLE patients also including selected cases.

METHODS

SLE-patients:

Those studied were 63 unselected patients (9 males, 54 females) and 39 selected patients (4 males, 35 females) with clinical diagnoses of SLE based on typical multisystem disease with positive ANA, all but two of whom fulfilled at least four of the 1971 ARA-criteria (10). They were followed from December 1980 through June 1983, attending the outpatient clinic every six to eight weeks, where 81 of them were regularly examined by two of the present authors (ON and GS).

The unselected patients constituted an epidemiological group, namely all known cases of SLE in the defined population of Lund's and Örup's health districts (11). The clinical features of the epidemiological patients are described separately (12). The aggregate follow-up period was 1366 months for the epidemiological group. The first six months of the prospective study in each unselected patient were used for matching with rheumatoid arthritis (RA) patients and controls (Table I).

When addressing hypotheses of relationships between infections on one hand and SLE parameters on the other hand, unselected and selected patients were handled as one SLE group. The distribution of fulfilled ARA criteria were comparable in the two groups (Table II), but the mean dosage of prednisolone per day was somewhat higher in the selected group (6 mg versus 4 mg). The aggregate follow-up period for all 102 patients was 2355 months.

TABLE I

COMPARISON OF MATCHED PATIENT GROUPS AND CONTROLS

	SLE	RA	Controls
Number	60	60	60
Males (%)	11.7	11.7	13.6
Median age (range)	45 (19-80)	47 (26-79)	46 (25-71)
Follow-up (months)	360	360	360
Without cortico- steroids (months)	186	204	360
Mean corticosteroid dosage (mg prednisolone/day)	2.8	2.7	0
Disease duration (years from diagnosis)			
median	4	14.5	
range	0 - 32	1 - 40	

Three of the 63 SLE patients could not be matched due to high corticosteroid dosage and were excluded when the groups were compared.

TABLE II

1982 ARA CRITERIA FULFILLED

Percent

CRITERION	UNSELECTED	SELECTED
	SLE	SLE
	n=63	n=39
Malar rash	43	59
Discoid rash	23	23
Photosensitivity	52	64
Oral ulcers	12	23
Arthritis	95	95
Serositis	54	59
Renal disorder	33	31
Neurological disorder	9	15
Haematological disorder	59	69
Immunological disorder	51	44
Antinuclear antibody	100	97

RA patients:

Sixty patients with rheumatoid arthritis (RA), including 41 with classical RA, 16 with definite RA according to ARA criteria (13) and 3 adults with juvenile onset of rheumatoid factor positive polyarthritis, were matched against the epidemiological SLE group with regard to sex, age and corticosteroid dosage (Table I). One SLE patient with impaired renal function was matched with an RA patient with amyloidosis and comparably reduced renal function. Since we were unable to match three of the SLE patients due to high corticosteroid-dosage, only 60 patients could be used for comparisons. The patients were followed for six months, from October 1982 to August 1983, in our specialized outpatient unit for chronic arthritis (Head: Merete Brattström, M.D.).

Controls:

Patients of the epidemiological SLE group were matched with regard to age and sex against 60 out of 75 healthy controls (husbands or wives of patients in the Dept. of Rheumatology), who agreed to participate in the prospective study and completed a questionnaire sent out after the six-month observation period, from January through June 1983 (Table I).

Registration of infection:

All patients and controls were instructed to report suspected infections without delay to permit immediate diagnosis. Infections were confirmed both by clinical findings and positive cultures, and by radiographic evidence or prompt regress in response to antibiotic therapy. Where bacterial isolates were not generally available, as in pneumonia, sinusitis or otitis, infection was confirmed by clinical findings in combination with either X-ray diagnosis (pneumonia and sinusitis) or regression in response to antibiotics.

Viral infections, generally upper respiratory infections, were considered to be present when the patient was found to

have acute incipient pharyngitis, rhinitis or gastroenteritis without positive bacterial cultures. Monitoring of viral infection was omitted in 21 patients not followed personally by the authors. Serology for 13 common viruses was performed in 12 patients with suspected viroses.

Organisms were designated as opportunistic according to Cohen's listing (14), which is based on principles proposed by von Graevenitz (15). Infections were considered to be major when accompanied by systemic manifestations such as fever or malaise and requiring systemic antibiotic therapy. An infection was registered when two or more organisms could be cultured from a given site. If supportive data were scant, or if doubt existed as to whether infection or clinical activity was the cause of actual symptoms, no infection was registered. Following Ginzler (6) incidence of infection or infection rate was expressed as the number of infections per 100 years of follow-up.

SLE-monitoring:

At each clinical attendance the clinical subset or organ system involvement and changes in disease activity were assessed without knowledge of immunological test results. The patients were assigned to clinical subsets according to the principles proposed by Lightfoot et al.(16), as modified by Sturfelt et al., and described separately (17); subsets were thus based on symptoms during the observation period. A vascular subset was defined by necrotizing vasculitis, severe Raynaud's phenomenon and cutaneous ulcers, or manifestations of regional ischemic disease as gangrene or myocardial infarction.

Intra-SLE group matching in the total material

When studying the influence of disease activity on the number of patients with infection, patients were used as their own controls. This was possible as, in the majority of cases, flares of activity persisted for only a few months.

In each case the control period chosen was the first period of inactive SLE which occurred during the prospective study, and which was of comparable duration to the period of active disease.

The relationship between SLE flares and infection was studied with reference to the first flare observed in each patient, by comparing the two months preceding peak activity with the two following months.

The observed periods of inactivity were used to study the influence of corticosteroid treatment on the number of infected patients. When a patient was observed during inactivity both with and without steroid-treatment, for statistical reasons only the longer of the two observation periods was used. Thus 48 patients were stratified to the group with corticosteroid treatment and 44 to the group without. With regard to leukocyte counts and serum levels of complement components patients were stratified in a similar manner to either of two groups respectively.

SLE-laboratory routines:

At each clinical attendance urine was analyzed and blood sampled for routine analysis of the complete blood picture, erythrocyte sedimentation rate (ESR), S-orosomucoid, SGOT, SGPT and S-creatinine. Complement components C4 and C3 were measured immunochemically (18), and C-reactive protein (CRP) was analyzed whenever infection was suspected. Serum and EDTA plasma (5mmol/l) were collected in aliquots and stored at -80 C for further analysis as necessary.

Data processing:

Data concerning past medical history, symptoms, physical findings, laboratory values, clinical assessment, therapy and registered infections were computerized, stored and handled with the aid of specially designed programmes in BASIC (Facit Desk Top Computer).

Statistics:

Chi-square analysis, McNemar test and median test were used when appropriate (19). Statistical analysis was restricted to comparison of observed frequencies in properly matched groups with equal observation periods.

RESULTS

A) SIX MONTHS CONTROLLED STUDY OF UNSELECTED SLE

Registered infections

During the six months prospective, controlled study 47 infections were registered in 32 of the unselected SLE patients. 21 infections in 18 patients were of bacterial origin, 1 fungal and 25 were suspected viral.

Among the RA cases, 37 infections were found in 33 patients, twelve infections being of bacterial origin and 25 of suspected viral origin.

Among controls a total of 33 infections occurred in 22 individuals, six infections being bacterial, one fungal and the remaining 26 suspected viral.

Comparisons of matched groups

The relative infection rates in the matched groups are shown in Table III. Lower urinary tract infections occurred with similar frequency in all three groups, both with regard to the incidence (Infections/100 years: SLE 17, RA 20, controls 10), and to the numbers of infected patients (SLE 5, RA 6, controls 3).

When lower urinary tract infections were excluded, the remaining number of patients with bacterial infections was greater ($p < 0.01$) in the epidemiological SLE group ($n=15$) compared with controls ($n=3$) but barely significant ($p < 0.06$, chi-square=3.69) compared with the matched-RA patients ($n=6$). The numbers of RA patients and controls with bacterial infections were comparable. Infections due to *S. aureus* were found only among the SLE patients. The infections encountered are seen in Table IV.

TABLE III

TOTAL NUMBER OF INFECTIONS PER 100 OBSERVATION YEARS

IN THE MATCHED GROUPS

Group	Viral	Bacterial	Fungal	Total
SLE *	83	53	3	139
RA	83	40	0	123
Control	87	20	3	110

* Unselected SLE patients

TABLE IV
INFECTIONS DURING 6 MONTHS IN MATCHED GROUPS
(Lower urinary tract infections excluded)

UNSELECTED SLE patients (n=60)	RA patients (n=60)	CONTROLS (n=60)
Paronychia *	Sepsis	Cellulitis
Pyodermia *	Bronchopneum.	Bronchitis
Abscess *	Sinusitis	Tonsillitis
Otitis media		
Bronchopneum.	Sinusitis	
Bronchitis	Tonsillitis	
Ulcer *	Apical osteitis	
Bronchitis		
Bronchopneum.		
Pyelonephritis		
Sinusitis		
Bronchitis		
Apical osteit.		
Bronchopneum.		
Osteomyelitis		
Bronchopneum.		

* Infection with S.aureus.

Fig 1

BACTERIAL INFECTIONS

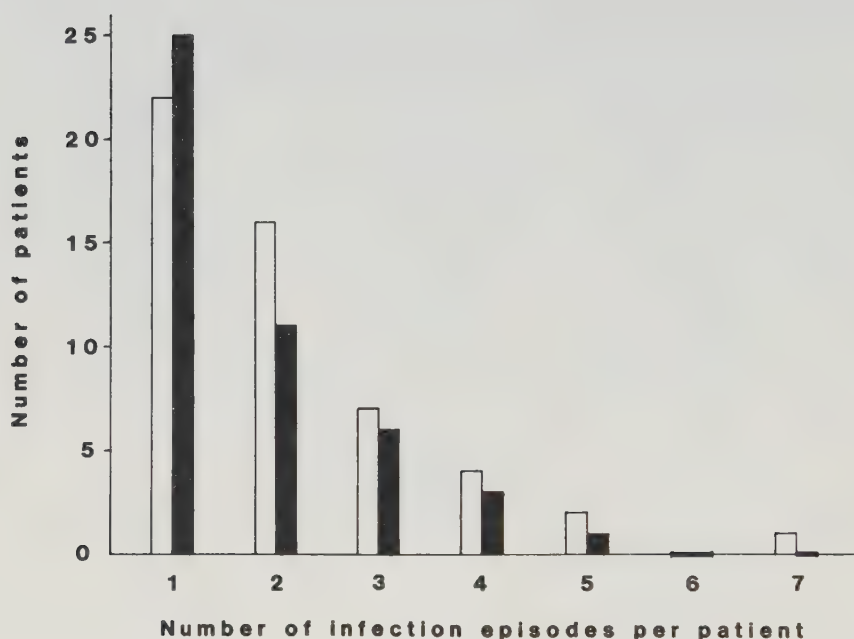


FIGURE 1

The distribution of all bacterial infections (open columns) in 52 of 102 SLE patients, and bacterial infections other than lower urinary tract infections (filled columns) in 45 of 102 SLE patients.

B) PROSPECTIVE STUDIES OF THE TOTAL SLE GROUPRegistered infections

A total of 240 infections, of which 162 occurred in the epidemiological group, were registered in 79 of the SLE patients studied from December 1980 through June 1983. 108 infections in 52 patients were of bacterial origin, 12 were fungal and 120 were suspected viral. The number of bacterial infection episodes per patient is shown in Figure 1. In the 81 patients followed by the authors, and in whom all infections were registered, suspected virosis

accounted for 61% of all observed episodes. In 9 of 12 analyzed, paired pilot-sera, from patients with suspected viroses, a fourfold rise in at least one viral titre was noted.

Bacterial infections

The incidences of the various bacterial isolates are specified in Table V. In five instances two organisms were cultured from a given site. In the epidemiological SLE group, 34 % of the bacterial infections were found in mucocutaneous tissue, 24% in the upper respiratory tract, 20% in the lower urinary tract and 13 % were bronchopneumonial.

TABLE V
ISOLATED BACTERIA FROM 102 SLE-PATIENTS

BACTERIA	TOTAL INCIDENCE	INCIDENCE IN UNSELECTED CASES
S.aureus	23	16
E.coli	17	6
Proteus spp	5	4
S.epidermidis	3	1
Beta hemolytic streptocc.	2	1
Enterobacter spp	2	2
Streptococc.faecalis	2	2
Acinetobacter	1	1
Alcaligenes faecalis	1	1
M.tuberculosis	1	0
Gram-negative (unspecif.)	1	0
TOTAL	58	34

TABLE VI

REGISTERED S.AUREUS INFECTIONS IN 102 SLE-PATIENTS

	Total number	Number in unselected cases
MAJOR INFECTIONS		
Sepsis	1	1
Pneumonia *	1	0
Sinusitis *	1	0
Gangrene infection	2	2
Osteomyelitis	2	2
Cutaneous abscess	3	2
Mastitis	1	0
MINOR INFECTIONS		
Ecthyma	6	3
Infected vasculitic ulcera	3	3
Traumatic wound infection	3	3
TOTAL	23	16

* = Positive nasopharyngeal culture indicating possible etiological organism.

Staphylococcal infections

Of 23 *S.aureus* infections among 16 SLE patients (Table VI),¹⁷ occurred in the epidemiological group. Of the eighteen integumentary infections, 9 were preceded by verified injury or other stress causing rupture of the skin. The three principal causes of skin disintegration were vasculitic ulcers, traumatic wounds or, in two cases, therapeutic arteriovenous (AV) fistulas. Of the two infections in the same patient due to AV fistulas, one was a sepsis with *S.aureus*. In two cases infection was superimposed on impaired peripheral circulatory function ultimately leading to amputation. The osteomyelitic infections required orthopedic removal of sequestra. Eleven of the 23 staphylococcal infections were classified as major infections.

Opportunistic infections

In 21 SLE patients 25 infections were found with organisms classifiable as opportunistic (Table VII). Six infections were seen in 5 of the epidemiologically retrieved patients, one among the RA patients and one in the control-group. The two latter infections both were vaginal candidiasis, the RA patient received 5 mg of prednisolone daily and the control was pregnant. One case of *S. albus* sepsis was seen in a selected SLE-patient with a central venous catheter during treatment for lupus pancreatitis with fever and thrombocytopenia. Two of the opportunistic infections were categorized as major. Among the minor infections were two cases of verrucal lesions, one of common warts on the hands, and one of venereal warts in the vulva; owing to their exuberant growth, these infections gave rise to appreciable psychological problems, and in the case of vulval warts a series of surgical excisions was necessary before they were successfully eliminated.

TABLE VII

OPPORTUNISTIC INFECTIONS IN 102 SLE-PATIENTS

INFECTION	MICROORGANISM	Total number	Number in unselected cases
MAJOR INFECTIONS:			
Miliary tuberculosis	M.tuberculosis	1	0
Sepsis	S.albus	1	0
MINOR INFECTIONS:			
Fever	Cytomegalovirus	1	0
Herpes zooster	Varicellae virus	5	0
Infected vasculitic ulcer	S.albus	1	0
Herpes simplex	Herpes virus	2	2
Verruca vulgaris		2	0
Condylomata acuminata		2	0
Oral thrush	C.albicans	3	0
Vaginitis	C.albicans	2	2
Erythrasma		2	1
Tinea	Trichophyton	4	1
TOTAL		26	6

Fig 2

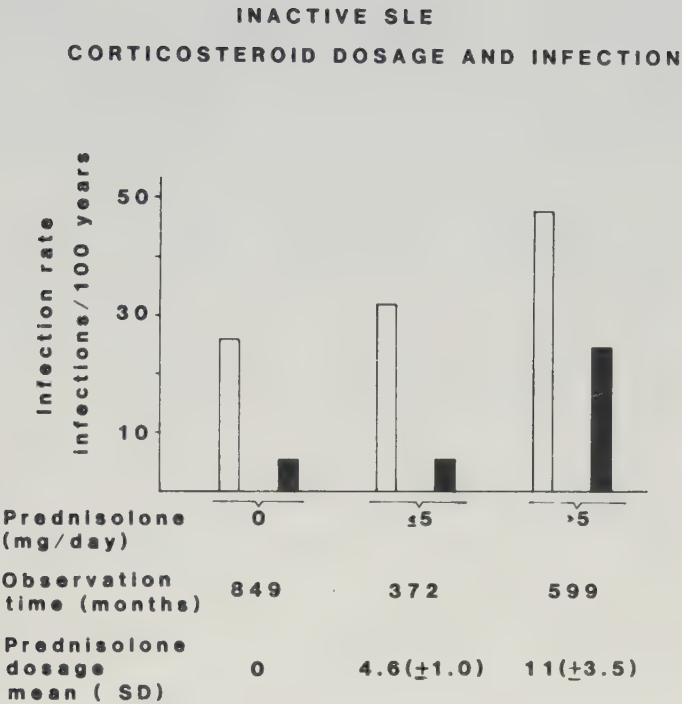


FIGURE 2

Calculated infection rates (Number of infections/100 observation years) of bacterial infections other than lower urinary tract infections (open columns), and opportunistic infections (filled columns) in a total of 102 SLE patients stratified in corticosteroid dosage subsets during periods without lupus activity.

Immunosuppressive therapy and infection

Nineteen bacterial infections other than lower urinary tract infections occurred during 849 observation months in 11 out of 44 inactive SLE patients without corticosteroid treatment. In 19 out of 48 inactive SLE patients with corticosteroid treatment, the corresponding finding was 31 bacterial infections during 849 observation months. The observed numbers of infected SLE patients were comparable ($p < 0.25$, chi-square=1.61). Calculated bacterial infections rates in different steroid dosage subsets are shown in Figure 2.

The 50 SLE patients without bacterial infections were retrospectively contrasted with the 10 SLE patients showing more than 2 bacterial infections when lower urinary tract infections were excluded (Fig.1 and Table VIII). Earlier prolonged corticosteroid therapy was significantly more common in the multi-infected group, but actual corticosteroid dosage and present lupus activity were comparable in both groups. Furthermore, 4 of the multi-infected patients belonged to the vascular subset, as defined in Methods, as compared with 2 of the non-infected group ($p < 0.01$).

Four opportunistic infections occurred in 4 inactive SLE patients without corticosteroid-treatment, and 10 in 7 inactive patients undergoing steroid treatment. Again, comparing the observed numbers of infected SLE patients, the difference was not significant ($p < 0.70$, chi-square=0.29). Calculated opportunistic infection rates in different corticosteroid dosage subsets are shown in Figure 2.

Nine patients were treated with azathioprine, a number too small for any conclusions to be drawn.

Clinical lupus activity and infection:

Fiftyseven bacterial infections other than lower urinary tract infections occurred in 32 inactive SLE patients during 1968 observation months, and 23 such infections were observed during 387 observation months with clinical disease

TABLE VIII

INFECTION AND PROLONGED CORTICOSTEROID THERAPY
IN 102 SLE PATIENTS

Group I = SLE patients with more than 2 bacterial infections during the observation period when lower urinary tract infections are excluded.

Group II= SLE patients without bacterial infections during the observation period.

	Combined median (CM)	Number of patients exceeding CM		Median test
		Group I	Group II	
	n = 60	n = 10	n = 50	
Years on corticosteroids	2	8	19	P<0.05
Months of activity under observation	1	6	22	P<0.70
Mg prednisolone dosage under observation	1	6	24	P<0.80

activity in 18 patients.

To eliminate the possible influence of corticosteroid therapy, the two patient groups were also stratified in two subsets, those with and those without steroid treatment. The patients served as their own controls (cf. Methods).

Thus 24 patients were observed for an aggregate of 103 months during SLE activity and without corticosteroid treatment. Of these patients, two had renal involvement, fourteen had articular or cutaneous manifestations, and eight had either serositis, fever or vasculitis. Since two patients had continuously active disease and thus could not serve as their own controls, 22 patients and an aggregate of 69 observation months were available for statistical analysis.

Correspondingly, 47 patients with lupus activity and simultaneously treated with corticosteroids were observed for an aggregate of 284 months, but since six of them had no periods of quiescent disease, 41 patients and an aggregate of 213 months of observation were available for statistical analysis.

The results are illustrated in Figure 3. The two subsets with active SLE both showed a significantly increased number of bacterial infected patients (Table IX).

Eight bacterial infections other than lower urinary tract infections, were observed for an aggregate of 55 months of activity, corresponding to the high rate of 175 infections per 100 observation years in seven SLE patients of the vascular subset.

Seventysix flares occurred in 49 of the SLE patients during the observation period. Of the 49 flares studied (the first in each patient), 7 were lupus glomerulonephritis, 18 were major extrarenal manifestations (neuropsychiatric, serositis or vascular), and 24 were minor flares (articular or cuta-

Fig 3

SLE

DISEASE ACTIVITY AND INFECTION

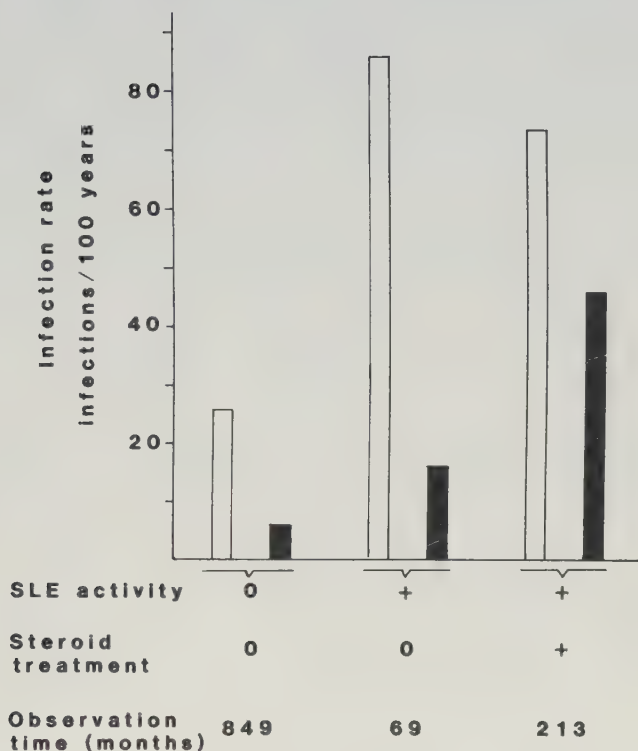


FIGURE 3

Calculated infection rates (Number of infections/100 observation years) of bacterial infections other than lower urinary tract infections (open columns), and opportunistic infections (filled columns) in a total of 102 SLE patients, stratified with regard to presence (+) or absence (0) of lupus activity and corticosteroid treatment. Mean prednisolone dosage per day in activity subset with steroids was 9.2 mg (SD=+/-4.8).

TABLE IX

INFECTIONS RELATED TO PERIODS OF SLE ACTIVITY
IN THE TOTAL SLE GROUP

Each patient serve as his own control

Infection	Steroid treatment	Number of patients infected only during		P*
		Period of activity	Control period	
Bacterial	0**	5	0	p<0.04
	+***	13	0	p<0.001
Opportunistic	0**	1	0	n.s.
	+***	7	0	p<0.01

* McNemar's test (19)

** Without corticosteroids: n = 22
observation time 2 x 69 months

*** Corticosteroid treated: n = 41
observation time 2 x 213 months

neous). Bacterial infections in connection with these flares are shown in Table X. The number of bacterial infections, other than lower urinary tract infections, increased significantly during the 2 months following peak activity of flares, compared with the period preceding peak activity. Five viral infections were observed before and eight following peak activity (n.s.). The infection rate before flares was comparable to the infection rate of inactive lupus in the total SLE group, both with regard to bacterial and viral infections.

TABLE X

INFECTIONS RELATED TO SLE FLARES

IN THE TOTAL SLE GROUP

(n = 49 patients with observed flares)

Number of SLE patients with (+) or without (-) bacterial infections before and after flares.

		< 2 months after	
		-	+
< 2 months	+	1	0
before	-	39	9

$P < 0.03$ (McNemar's test)

Sixteen opportunistic infections occurred in 15 patients with inactive SLE, and eleven in 10 patients with active SLE. When opportunistic infections were calculated in the subsets with and without corticosteroid-treatment(Fig.3), a significantly increased number of infected patients was found during the test period in the subset with active lupus and steroid therapy (Table IX).

Uremia and infection rate

Only two SLE patients were uremic and both started dialysis during the observation period. One of these patients had two bacterial infections, the other had one infection; S.aureus caused two of the infections, one of which was a sepsis. The estimated bacterial infection rate per 100 observation years was 180, but in this case the calculation was based on an observation period of only 20 months.

Leukopenia, hypocomplementemia and infection rate

In patients with leukopenia ($p < 0.025$), or hypocomplementemia ($p < 0.025$), no increase of bacterial infections was found but a tendency towards increase of opportunistic infections was noted (Table XI).

Hospitalization due to infection

Twenty-eight of the 63 SLE patients in the epidemiological group were hospitalized for an aggregate of 1277 days during 1981 and 1982 on 63 occasions (Median duration 8 days/occasion). Twelve of the admissions were partly motivated by infection, and resulted in an aggregate of 591 days in hospital. In the contrast group of 60 RA patients 29 were hospitalized for an aggregate of 887 days on 53 occasions (Median duration 13 days/occasion) during the two years studied. Three of the admissions in the RA group were caused by infection, and resulted in 168 days in hospital. Data on hospitalization in controls are not available.

Of the 81 SLE patients sequentially followed by the authors, 29 patients were hospitalized for an aggregate of 1355 days

TABLE XI

LEUKOCYTE COUNTS AND SERUM LEVELS OF COMPLEMENT
COMPONENTS (C3 and C4) RELATED TO THE NUMBER OF
INFECTED SLE PATIENTS

Complete sequential laboratory data available in 79 of
102 SLE patients

	C3 and/or C4		Leukocyte counts	
	Reduced	Normal	Reduced	Normal
Patients				
number	31	48	27	52
months observed	337	337	172	172
Infected patients				
bacterial	7	12	5	8
opportunistic	7*	3	4**	2

* n.s. ($p < 0.08$, chi-square= 3.19)

** n.s. ($p < 0.20$, chi-square= 1.68)

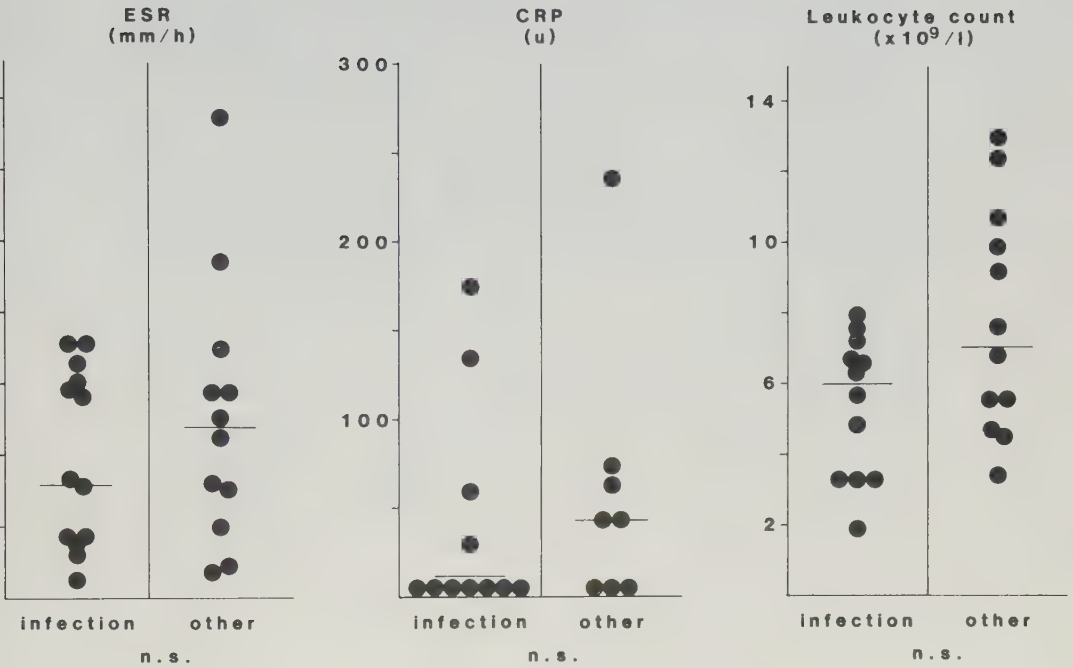
during 1981 and 1982 on 56 occasions. Twentyfour of the 56 hospitalizations (43%), occurring in 16 patients, were due to a preliminary diagnosis of infection, infections being verified in 10/24 cases (42%). Twelve occasions were diagnosed as lupus activity without infection, and two were drug--fever induced by antibiotics started in primary care (Trimethoprim in a case of lower urinary tract infection and klindamycin in a case of mastitis). Three additional infections, not initially suspected but with symptoms appearing during hospitalization, were diagnosed among the 32 occasions without a preliminary infection diagnosis.

Initial fever was associated with infection in 6/17 cases (35%). Neither laboratory findings such as ESR,CRP and leukocyte counts (Fig.4), nor prednisolone therapy distinguished patients with infections later verified from those without infections. Only repeated microbial cultures were reliable for diagnosis.

Mortality in infections

Of the seven patients with SLE who died, five belonged to the epidemiological group. The fatalities being due to sepsis in two cases, one caused by E.coli and the other by S.aureus. The course in one young female with a clinical diagnosis of SLE was complicated by acute lymphatic leukemia and she died in a gram-negative sepsis due to granulocytopenia induced by cytostatic therapy. Two cases,one myocardial infarction and one cerebrovascular disease, were complicated by bronchopneumonia probably contributing to the fatal outcome. No infections were detected in the two remaining cases, one myocardial infarction in a young woman and one oral carcinoma. Four of the infected deceased patients had vascular subsets of SLE.

LABORATORY TEST



DIAGNOSTIC OUTCOME

FIGURE 4

Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and leukocyte counts on 27 hospital admissions of SLE patients where infection was initially suspected in 24 cases and subsequently diagnosed in 10 with and 3 without a preliminary infection diagnosis. Results of all laboratory tests were not available in all cases. Bars indicate medians. Significance tested with median test.

DISCUSSION

Here we present the first prospective and controlled clinical study of infections in an epidemiologically retrieved SLE-population. This unselected population includes many mild cases, with a low overall level of corticosteroid treatment. The circumstance of periods of clinical active SLE not treated with corticosteroids enabled us to determine lupus activity in relation to infection. Moreover, the regularly spaced check-ups regardless of disease activity and the standardized registration of infection made possible fairly thorough identification of clinical infections in the groups under study. This is reflected in the viral and bacterial infection rates, which were, respectively, three and two times those reported by Ginzler(6). Although the controls were not followed in the outpatient clinic, the close similarity between the SLE, RA and controls with regard to suspected viral infection rates reflects homogeneity of infection registration among the three groups. Furthermore viral serology, when performed, indicated in 75 % of the cases one causing virus out of the 13 tested.

In agreement with Staples (8) we found a significant increase of bacterial infection rate in SLE. We could not, however, find any increase of viral or fungal infections when we compared SLE patients with matched controls or with matched patients with RA, in contrast to the proposal by Ginzler (6). Probably this discrepancy is partly due to a lack of matched controls in previous studies and partly to the unselected patient population in our study. We were unable to confirm the tenfold increase in infection rate in SLE compared with RA described by Staples (8), a discrepancy that may perhaps be accounted for by the lack of matching and the selection of in-patients with hospitalization times exceeding 4 weeks in Staples work. Furthermore, the rate of lower urinary tract infections were comparable in all three matched groups of our study, a finding not reported by others,

and probably indicating a separate mechanism of first line defense in the lower urinary tract.

That the rate of S.aureus infection was significantly raised in our SLE patients is in keeping with clinical experience (16,20) and has focused interest on opsonization of S.aureus in SLE-sera (21,22). The importance of skin traumatization for S.aureus infection has been reported in other diseases (16).

Bacterial infections probably occur at an increased rate even in RA (23), especially in cases with prominent systemic disease manifestations. One explanation for the relative absence of bacterial infections in our RA group may be that, owing to the age matching, the group comprised younger patients, less likely to suffer from severe extraarticular manifestations. Again, owing to the nature of the disease among RA patients, there may have been a greater probability of their disease being quiescent during the period under study.

A noteworthy aspect of this study is that SLE and RA patients were also matched for corticosteroid dosage, which indicated a steroid-independent rise of bacterial infection rate in lupus. Although suspected in earlier reports (6,8), this has not previously been shown in controlled prospective studies.

The high infection rate found in the vascular SLE subset does not appear to have been reported earlier. The sites of bacterial infections do, however, correspond rather well to Ginzlers findings (6) indicating the possibility of similar mechanisms behind the infections.

Prolonged corticosteroid therapy leading to skin atrophy (24) and eventually atherosclerosis (25,26) might together with dermal vasculitis, Raynaud's phenomenon, chronic ulcers or peripheral gangrene (27) provide the preconditions for

increased access by pathogenic micro-organisms in patients with vascular subsets. Our retrospective findings in multi-infected patients with earlier prolonged corticosteroid therapy, and the high prevalence of a vascular subset in these patients, would appear to support such a hypothesis, though the retrospective design may have included other, undetected differences between the groups.

Another mechanism, possibly responsible for an increase in the number of infections in these vascular subsets, might be circulating immune complexes in active SLE that, by reducing opsonic capacities (22), also impair phagocytic defense in these patients.

The possible prognostic significance of the vascular subset is illustrated by the high mortality among these patients in our study. Unlike the cardiovascular fatalities, without signs of SLE activity or infection, reported by Urowitz (28), the vascular fatalities among SLE patients studied here were often combined with infections. A hypothesis of accelerated atherosclerosis in SLE could, however, be proposed for both studies.

A hypothesis often cited is that lupus activity is triggered by infection (9), and has found analogous pathogenetical support in Goodpasture's syndrome (29) and Wegener's granulomatosis (30) and clinical support in Systemic Juvenile Chronic Arthritis (31). However, this hypothesis derives no support from our study, in which the situation was reversed, with an increased bacterial infection rate following increasing lupus activity, a circumstance in accord with the proposals of Staples and Ginzler (6,8). Naturally, this does not rule out the possibility of microbial tissue injury initiating local immunological events preceding lupus activity in individual cases, an eventuality which might explain the cases found in the literature (32).

Interestingly, periods with immunochemically determined hypocomplementemia were not related to increased rates of bacterial infections with common pathogens in the present work, in contrast to earlier reports (6). Nor did leukopenia, chiefly neutropenia, increase the rate of infections with common pathogens.

The correlation between infections on one hand, and corticosteroid treatment (33) and corticosteroid dosage (6) on the other, could not be verified by our results, probably due to the fact that no cases treated with high-dose regimens were represented in our series.

Only a marginal increase of opportunistic infection rates was found in the epidemiological patient group. However, a substantial number of such infections were accumulated when the referred SLE patients were added, in agreement with previous studies on selected groups (6). Steroid treatment, rather than disease activity, correlated to opportunistic infection in the total SLE population under study.

Suspected infection was a common cause of hospitalization and it was noted that repeated microbial cultures were required before a definite diagnosis could be made. Thus the measurement of C-reactive protein (CRP), for instance, failed to support the hypothesis that SLE patients produce more CRP in response to infection than to lupus inflammation, as has often been claimed (34,35), though more recently this has been challenged (36). The proportion of hospitalized patients with fever, and in whom infections were later confirmed, is in agreement with Stahl's findings (23%) in his retrospective analysis of fever in SLE (37).

In conclusion, our data indicate the importance of bacterial infections in SLE, necessitating a greater awareness of disease-related and therapeutical factors shown to increase sensitivity to infections. For example, uncontrolled lupus activity, especially of the vascular subset, and traumatiza-

tion of the skin seem to be of importance. High corticosteroid dosage is probably one of the most important factors to be considered (6,8), although this could not be verified with the low dose series studied here. Better diagnostic tools are needed, to identify infections in acutely ill SLE patients. The case for proper diagnosis is further stressed by the fact that both antibiotic and corticosteroid treatment constitute potential risks in SLE (38,39,40), and therefore should preferably be used only when explicitly indicated.

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Reduced opsonisation of protein A containing *Staphylococcus aureus* in sera with cryoglobulins from patients with active systemic lupus erythematosus

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SUMMARY Among a total of 41 patients with systemic lupus erythematosus (SLE) 11 of 14 patients with active disease had reduced capacity ($p<0.05$) to opsonify *staphylococcus aureus* in undiluted sera, as compared with nine of 27 patients with inactive disease ($p<0.02$). The opsonic reduction in the active patients increased with the number of active organ systems ($p<0.002$). No correlation was found between reduced opsonisation and corticosteroid treatment, or serum concentrations of complement components (C) of the classical pathway, or bacteria-associated activated C3. When the cryoglobulin fraction of immune complexes (IC) was removed, normal opsonic capacity was restored, and the opsonic reduction could be transferred with the cryoglobulins to pooled serum. Increased IC values, as measured by C1q binding assay, were found in conjunction with reduced opsonic capacity ($p<0.04$). Since opsonisation in SLE sera of a protein A deficient strain of *S. aureus* was normal, reduced *S. aureus* phagocytosis in SLE sera may be explained by IC binding to staphylococcal protein A.

Key words:

In a recent prospective and controlled study we have shown an increase of bacterial infections, especially those caused by *S. aureus* (unpublished data), in an unselected group of SLE patients from a defined population.¹ The infectious episodes occurred predominantly during periods of disease activity.

Phagocytic killing is considered to be the major host defence against bacterial infections.^{2,3} In sera from patients with SLE impaired phagocytosis and microbicidal capacities have been reported for normal phagocytes.^{4,5} Phagocytic cells identify an opsonised prey and attach it to their cell membranes with the aid of receptors for the Fc fragment of immunoglobulin G and the CRI receptor for activated C3.⁶ Defective opsonisation due to reductions of C and correlating with infections in active SLE has been suggested as one possible mechanism behind impaired phagocytosis.^{4,7,8}

Serum concentration and opsonisation time are of critical importance to results when opsonin func-

tions are studied.⁹ In this study we present a modified assay system with short-time opsonisation in undiluted serum. We used as test organism a protein A containing strain of *S. aureus*,¹⁰ which is opsonised by 'natural' antibodies to peptidoglycan in the presence of C of the classical pathway.¹¹⁻¹³ With this system reduced opsonisation was found in some SLE sera. The mechanisms behind the reduction were studied, and the results were correlated with the current clinical status of the patients, in conjunction with blood sampling.

Patients and methods

Informed consent was obtained from the blood donors and the study was approved by the Ethics Committee of the University of Lund.

PATIENTS

From a sequential study of SLE patients¹⁴ a series of 41 consecutive patients were included in the present study. All 41 patients fulfilled at least four of the 1971 American Rheumatism Association (ARA) criteria¹⁵ or four of the 1982 ARA criteria.¹⁶ The

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median age was 37 years (range 18–71 years), and four patients were males. At intervals of 6–8 weeks the patients were followed up prospectively between 1980 and 1983 in a lupus clinic of the Department of Rheumatology. At each visit disease activity was clinically assessed according to the principles proposed by Lightfoot and Hughes¹⁷ and modified by Sturfelt *et al.*^{14, 18} Infections were monitored as part of a study of infections in SLE (unpublished data).

PHAGOCYTES

100 ml of blood was drawn from a cubital vein of healthy members of the hospital staff. The blood was immediately defibrinated by gentle rotation for 10 minutes in a flask containing glass beads. A modified Boyum technique¹⁹ was used in the following manner. The defibrinated blood was sedimented at room temperature with an equal volume of 2% dextran T 500 (Pharmacia, Uppsala, Sweden) in isotonic sodium chloride. After 45 minutes the leucocyte-rich supernatant was withdrawn, and 25 ml was layered on top of 15 ml of an Isopaque-Ficoll mixture in each of four 30×115 mm plastic tubes. (Falcon, Oxnard, California, USA). The tubes were centrifuged with a swing out rotor for 15 min at 1350×g. The upper cell layer containing monocytes was pipetted off into two tubes and centrifuged to a pellet. The granulocyte pellets from two tubes were each suspended in 7 ml of the original serum-dextran mixture and gently mixed for 5 min with 35 ml of 0.87% ammonium chloride for erythrocyte lysis. After centrifugation the pellets were washed twice in 40 ml of Parker's fluid 199 (National Bacteriological Laboratory, Stockholm, Sweden) and suspended in the incubation medium which consisted of 10% heat-inactivated pooled serum in Parker's fluid 199, or Parker's fluid 199 only. The cells were counted in a Bürker chamber, and their final concentration was adjusted to approximately 5×10^9 phagocytes/l incubate.

SERUM

Pooled serum was collected from 34 healthy volunteers. Individual sera were collected from 15 healthy members of the hospital staff, seven of the sera being used for determining the variance of single sera from one subject to another.²⁰ Blood was sampled from the 41 SLE patients according to the SLE control programme of the series of patients under consideration here. Sera collected at 4°C²¹ were used for complement analysis, and sera collected at 37°C²² were used for opsonic studies and cryoglobulin isolation. Fifty-nine sera, usually including the first available sample from each patient during the prospective study, were analysed by the opsonic assay. Serum from one patient in a family

with fulminant meningococcal infections and hereditary properdin deficiency, less than 1% of normal,²³ was kindly provided by Dr J H Braconier, Department of Infectious Diseases, University Hospital, Lund.

BACTERIA

Staphylococcus aureus (strain 502 A) was used as the test organism. In some experiments separately reported under 'Results' tests with *S. aureus* (strain Wood 46) were run in parallel. Bacteria were cultured overnight in Bacto antibiotic medium 3 (Difco, Detroit, USA), twice washed in isotonic sodium chloride, and then opsonised.

OPSONISATION

The washed bacterial pellet was suspended in 1 ml serum (undiluted) or 0.1 ml serum in 0.9 ml Parker's fluid 199 (diluted serum) and incubated at 37°C for either 3 or 30 minutes while being gently agitated. Opsonisation was arrested by the addition of 0.01 M ethylenediaminetetra-acetic acid (EDTA). The opsonised bacteria were washed twice in isotonic sodium chloride and adjusted to an optical density of 0.23 at 618 nm. After dilution in Parker's fluid 199 a final bacterial concentration of approximately 40×10^9 colony forming units/l was achieved.

FIXATION OF C3

Anti-C3d rabbit IgG (antihuman C3d complement Dakopatts a/s, Copenhagen, Denmark) was labelled with ¹²⁵I (100 mCi/ml, Amersham, Buckinghamshire, England) by the chloramine T method.²⁴ *S. aureus* 502A were incubated for 3 min in undiluted patient or control sera in a total volume of 0.5 ml. Opsonisation was stopped by addition of 10 ml isotonic saline at 4°C, and the bacteria were washed twice and adjusted to an optical density of 0.23 at 618 nm. After dilution in Parker's fluid 199 to a bacterial concentration of 10^9 bacteria/l, 50 µl labelled anti-C3d (250 000 cpm) in Parker's fluid 199 was added. After 15 min at 37°C with the labelled antibody the bacteria were washed in Parker's fluid 199. The radioactivity in the bacterial cell pellet was measured (Gamma Sample Counter, LKB, Stockholm, Sweden). The results were expressed as a percentage of the counts per minute in the reference (pooled) sera. In C2-deficient or heat-inactivated sera, the counts per minute were reduced by more than 80%.

BACTERIAL KILLING

A modification of the Maaloe technique²⁵ was used for bacterial killing. 50 µl of the bacterial suspension was added to 0.2 ml of the cell suspension in 12×75 mm plastic capped tubes (Falcon, Oxnard, Califor-

nia, USA). Incubation was at 37°C, while the tubes were gently agitated in a vertical position. Incubation was stopped after 15 min by adding 2 ml distilled water containing 0.01% bovine serum albumin and 1.5% Triton X 100 (isooctylphenoxypolyethoxyethanol) at 4°C, and the suspension was allowed to stand for 3 min to permit cellular lysis in the tube. 0.2 ml of the suspension was then diluted in 7.8 ml distilled water containing 0.01% bovine serum albumin. Viable bacteria were determined by colony counting by the pour plate method. The process for cellular lysis did not affect bacteria viability.

When normal pooled sera or SLE were heat inactivated, opsonisation did not result in bacterial killing, either by granulocytes or by monocytes. Nor did opsonisation in sera treated with a chelating agent (EDTA) support bacterial killing. Neither 1 mg/l nor 20 mg/l prednisolone (Precortalon aquosum) affected either granulocyte or monocyte killing. The rate of killing was linear for 60 min when plotted on a linear-log scale. Incubations were always performed in duplicate. Controls without cells were always included, and no deaths occurred.

COMPLEMENT COMPONENTS

The complement components (C) C1q, C1s, C3, and C4 were quantified in all sera by electro-immunoassay.^{26, 27} All sera were screened by haemolysis in a gel functional complement assay which detected deficiencies in the classical and alternative pathways of complement.²⁸ When pooled serum was incubated at 37°C and C analysed at two-hourly intervals, a reduced lysis of the classical pathway appeared 16 hours after the start of incubation.

CIRCULATING IMMUNE COMPLEXES

Circulating immune complexes were measured by a fluid phase C1q binding assay (C1q BA) as described by Zubler *et al.*²⁹ The results of C1q BA measurement in nine SLE sera collected both at 4°C and 37°C were comparable for the two temperatures.

CRYOPRECIPITATION

Cryoprecipitation was performed according to Svensson *et al.*,³² with serum collected at 37°C and immediately transferred to a water bath for clotting at 37°C for 2 h. The serum was then stored at 4°C for four days as suggested by Cream.³⁰ The resulting cryoprecipitate was spun down at 4°C, the supernatant was pipetted off and stored, and the pellet was redissolved at 37°C in a corresponding volume of pooled control serum. Native SLE serum, super-

natant of SLE serum, and pooled serum with dissolved cryoglobulin, each in a volume of 0.5 ml, were collected from nine SLE patients. Five of the patients had reduced opsonic capacity ($p < 0.05$), while four were within the normal range.

STATISTICS

When calculating the significance of differences between groups of independent values Student's *t* test was used for normal distributions. The non-parametric χ^2 test for two independent samples and Spearman's rank test were used, as appropriate.³¹ Opsonic bactericidal capacity was rated according to a leucocyte bactericidal index (BI), based on the Student paired *t* test, after Hoffman and Bullock.²⁰ The following formula was used:

$$BI = \frac{\log \% \text{ viable control} - \log \% \text{ viable SLE}}{\text{normal 'between-subject' SD}}$$

The normal 'between-subject' standard deviation (SD) separately determined for monocytes and granulocytes was 0.11 for both phagocytic cell lines.

Results

EVALUATION OF OPSONIC ASSAY

Opsonisation in undiluted normal pooled serum for 3 min produced significantly higher phagocytic killing of *S. aureus* than did bacterial opsonisation in 10% serum for 3 or 30 min and was comparable to bacterial opsonisation in undiluted serum for 30 min. The observed killing capacities of granulocytes and monocytes were comparable (Fig. 1).

Bacterial opsonisation for 3 min in undiluted, C-deficient patient sera is shown in Fig. 2. Opsonic capacities were reduced in sera from two SLE-patients with hereditary C2 deficiency, and in sera from one patient with pronounced hypocomplementaemia, probably genetically determined, of C4. The sera from one of the two C2-deficient patients did not have increased concentrations of immune complexes. Opsonisation in sera from a patient with inherited properdin deficiency on the other hand was within the normal range.

OPSONISATION IN SLE SERA

Opsonisation was studied in 41 sera, i.e. one sample from each patient included in the sequential study; 14 samples were collected during disease activity and 27 samples during quiescent disease. Opsonic capacity was reduced ($p < 0.05$) in 11 of the 14 'active' sera, as compared with nine of the 27 'quiescent' sera ($p < 0.02$). Table 1 shows the SLE symptoms in the 14 patients with active disease and the granulocyte bactericidal index (GBI). A correlation exists between the number of active organ

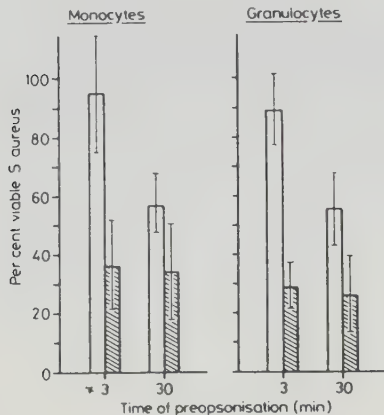


Fig. 1. Phagocytic killing of *S. aureus* by monocytes and granulocytes. Opsonisation of 150×10^6 *S. aureus*/L was performed for 3 or 30 min with 10% (open columns) or undiluted (hatched columns) normal pooled serum. Incubations were performed for 15 min. The bars indicate SD of five or 13 (undiluted serum, 3 min) experiments. Opsonisation in 10% serum was less effective than opsonisation in undiluted sera when 3 min ($p < 0.001$) and 30 min (monocytes $p < 0.05$, granulocytes $p < 0.01$) opsonisation times were compared.

systems and the reduction of opsonic capacity ($p < 0.002$, Spearman's rank test). Six of the 11 patients with reduced in-vitro opsonisation had moderately reduced ($p < 0.025$) C concentrations (C1q, C3 or C4), and eight had increased ($p < 0.05$) IC concentrations (as measured by C1q BA).

In eight cases patient sera were studied both when SLE was clinically active and when it was quiescent. In all patients (one patient being C2 deficient) the opsonin capacity of their sera was reduced in active disease, as compared with that in quiescent disease ($p < 0.05$).

Opsonic capacity was reduced in four out of five sera sampled within the two-month period preceding bacterial infection, as compared with nine out of the remaining 36 sera with no infection within two months ($p < 0.05$). Two of the four predicted infections were deep major infections caused by *S. aureus*, and one was a septicaemia with *S. albus*. In six patients sera were collected during infection but before antibiotic treatment was started, and opsonic capacity of sera was within the normal range in all six sera.

MECHANISM OF REDUCED OPSONISATION IN SLE SERA

Reduced opsonisation ($p < 0.05$) with the present method was found in 20 of the 41 SLE patients. Ten of the patients with reduced opsonisation were receiving corticosteroid treatment (mean dose 10 mg/day), compared with 12 of the patients with normal opsonisation (mean dose 12.5 mg/day). These results do not indicate an influence of corticosteroid treatment on the opsonisation results. Fig. 3 shows the simultaneous granulocyte and monocyte killing of *S. aureus* 502 A resulting from 22 of the experiments. Granulocyte and monocyte killing of bacteria opsonised in the same SLE sera were similarly reduced.

Concentrations of C determined from sera with reduced opsonic capacity and from sera with normal opsonic capacity showed no significant differences (Table 2). However, increased concentrations of

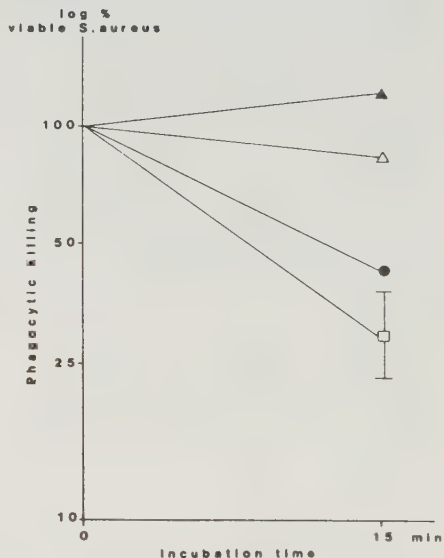


Fig. 2. Reduced phagocytic killing by granulocytes of *S. aureus* opsonised for 3 min in undiluted sera from two SLE patients deficient in complement component 2 (▲, $p < 0.001$), one SLE patient deficient in complement component 4 (△, $p < 0.05$), one healthy individual with properdin deficiency (●, not significant) compared with a normal pool (□). The results are means of duplicate experiments and bars indicate 'between-subject' SD.

Table 1 SLE symptoms (GBI)

Patient	SLE symptoms	GBI
1*	Pancreatitis, leucopenia, thrombocytopenia, fever	-12.3†
2	Pericarditis, malar rash, cytoid bodies, fever	-5.5‡
3	Lupus nephritis, exanthema, fever	-4.8‡
4	Pleuritis, malar rash, fever	-3.4‡
5	Arthritis	-3.2‡
6	Malar rash, arthritis	-2.9§
7	Lupus nephritis	-2.9§
8	Lupus nephritis, CNS symptoms, cardiomyopathy, malar rash	-2.7§
9	Arthritis	-2.5§
10	Myelitis, fever	-2.4§
11	Myelitis	-2.4§
12	Carpal tunnel syndrome	-1.6
13	Myocardial infarction	-1.6
14	Arthritis	0.6

*Homozygous C2 deficiency. GBI during inactive SLE = -5.5.
 † $p < 0.001$. ‡ $p < 0.01$. § $p < 0.05$.

circulating IC were found in conjunction with reduced opsonic capacity (Table 2).

Labelled antibodies to C3d showed that the bacteria-associated C3d was comparable in nine sera

with reduced opsonic capacity (median 68%, mean 82%, SD $\pm 23.6\%$, to that in five sera with normal opsonisation (median 83%, mean 82%, SD $\pm 13.2\%$). The C3d concentrations observed showed no correlation with opsonisation as determined by granulocyte killing of bacteria (Spearman's rank test, $r = -0.04$).

The opsonic capacities of nine SLE sera from which cryoglobulins had been eliminated were compared with those of the nine corresponding native SLE sera. When the cryoglobulin fraction of IC was eliminated opsonic capacity was restored to normal. When bacterial opsonisation was simultaneously performed in nine pool sera, to each of which had been added one separated SLE cryoglobulin, and the results were compared with those of native pool sera, it was found that the opsonic reduction, or increase, could be transferred with the cryoglobulins to pooled sera. The relationships between the two differences calculated from each cryoglobulin elimination are shown in Fig. 4.

The possible role of staphylococcal protein A in opsonisation was studied with the protein A deficient strain *S. aureus* Wood 46, which was opsonised in parallel with *S. aureus* 502 A in six SLE sera. Five sera showed reduced opsonic capacities for *S. aureus*

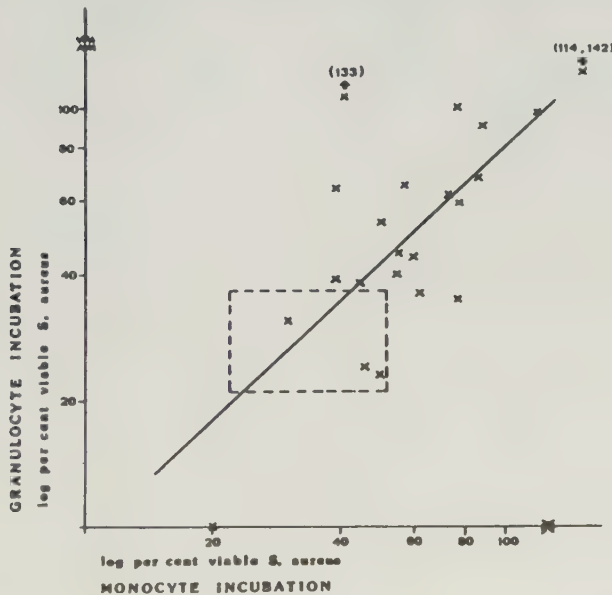


Fig. 3 Simultaneously determined killing capacities of normal monocytes (abscissa) and granulocytes (ordinate) when incubated for 15 min with *S. aureus* opsonised in undiluted SLE sera ($n = 22$, $r = 0.56$, $p < 0.01$, Spearman's rank test). The dotted box indicates one standard deviation when opsonisation was performed in undiluted normal sera ($n = 13$).

Table 2 Complement components (C) and circulating immune complexes (IC) in sera from SLE patients with normal or reduced opsonic capacity

	Number of patients with reduced ($p<0.025$) C or increased ($p<0.05$) IC concentrations		p
	Normal (n=21)	Reduced (n=20)	
C1q	2	6	NS
C1s	1	2	NS
C4	3	8	NS
C3	2	6	NS
IC	10	17	<0.04

NS=not significant.

502 A, whereas opsonisation of *S. aureus* Wood 46 was within the normal range in all cases (data not shown).

Discussion

We have described a modified method for determining heat-labile opsonin capacity in non-diluted serum and its application to SLE sera.

Most earlier methods are based on the use of diluted sera for opsonisation.^{8, 32-34} Dilution might

introduce pitfalls, for example, the alternative complement pathway might be more sensitive to dilution than the classical complement pathway.³⁵ Furthermore dilution affects both time-dependent and concentration-dependent kinetics.⁹ With our method sources of error due to serum dilutions or to time factors were minimised.

Our finding of an almost identical response to opsonisation of *S. aureus* in both monocytes and granulocytes is in agreement with a recent study on the opsonisation of *S. pneumoniae*.³⁶ Earlier findings of a slower rate of monocyte ingestion³⁷ could not be reproduced with our assay technique.

The need for activation of the classical complement pathway to opsonise *S. aureus* 502 A¹³ is in agreement with our findings of highly reduced opsonisation in sera from patients with hereditary C2 deficiency and of chiefly normal opsonic capacity in serum from a patient with a hereditary properdin deficiency.

The finding of reduced opsonisation in clinically active SLE is in keeping with our recent report of increased frequency of bacterial infections in patients with active disease. Furthermore the correlation, found in this study, of opsonic reduction with the number of active organ systems parallels our clinical finding that bacterial infections are more

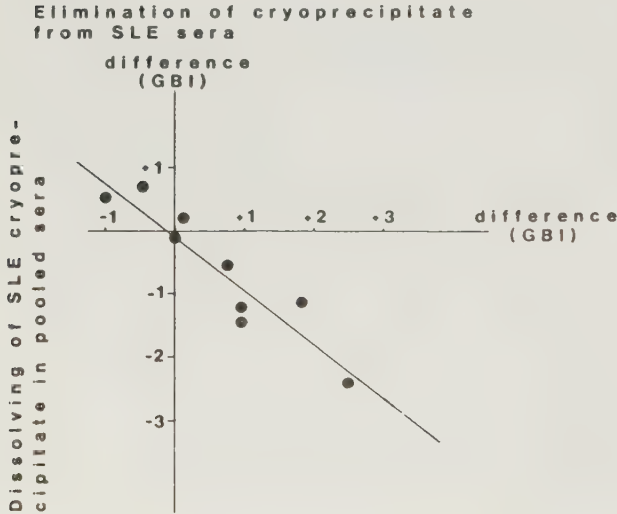


Fig. 4 Changes of granulocyte bactericidal index (GBI) between opsonisation in native SLE sera and in supernatant (with the cryoprecipitate eliminated) were comparable with changes of GBI between opsonisation in pool sera and in pool sera in which the corresponding SLE cryoprecipitate had been dissolved (n=9, $r=0.91$), $p<0.01$ Spearman's rank test). Opsonisation time was 3 min and the sera were undiluted.

common during peak activity of lupus flares (unpublished data).^x

Although based on limited material, the clinical importance of reduced opsonic capacity is suggested, since bacterial infections are more common in the two months after reduction of opsonisation than when opsonic capacity was normal. Our observation of normal opsonic capacity during infection is in contrast to earlier findings,⁴ probably due to the fact that the few days interval between onset of infection and blood sampling in our study allowed activation of host defence mechanisms.

Jasin showed that reductions of complement components correlated both with reduced opsonic capacities and with infections in active SLE. Almost all of Jasin's patients (27/30) had reduced complement concentrations,⁴ whereas half of ours did not – a discrepancy presumably reflecting the different populations under study, our patient group probably having less renal disease, as hypocomplementaemia is predominantly seen in active SLE with renal involvement.²¹⁻³⁸ Nevertheless in this series the proportion of patients with reduced opsonisation during active disease is higher than the corresponding value (12/30) reported by Jasin.⁴ We found that heat-labile dependent opsonic capacity was reduced even when complement concentrations were not, this may be due to the use of undiluted sera in our assay technique, or to our blood sampling at 37°C. When radiolabelled antibodies to C3d were added the amounts of bacteria-associated activated C3 after opsonisation in SLE sera were comparable to those of the controls, indicating that defective opsonisation in SLE sera cannot be explained by reduced bacterial C fixation.

Our results thus indicate the possibility of some factor in SLE sera inhibiting either Fc or CRI receptor contact on the phagocyte. SLE is the prototypic IC disease in man³⁹ and IC might be a blocking factor in our assay by binding to protein A of the bacterial cell walls through the Fc portion of its IgG.⁴⁰⁻⁴¹ Protein A has actually been used to isolate IC⁴² and to assay IC in SLE sera, with results paralleling those of a C1q BA.⁴³

When an IC fraction was eliminated from our SLE sera by cryoprecipitation, opsonisation was restored, thus indicating the involvement of IC in the reduced opsonic capacity of SLE sera; and the finding that reduced opsonic capacity could be transferred to normal pooled sera by the cryoprecipitate provides further support for a hypothesis of unspecific IC-mediated reduction of opsonisation. The correlation between increased concentrations of IC as measured by C1q BA, and reduced opsonisation is additional, indirect evidence for IC involvement. Our results with normal opsonisation of the *S.*

aureus Wood 46 strain, which has been shown to be protein A deficient,¹⁰ in SLE sera with low opsonic capacity for *S. aureus* 502 A, also indicate the involvement of IC interaction with protein A in defective phagocytosis.

Thus we have found that reduced in-vitro opsonisation of *S. aureus* in undiluted SLE sera in clinically active lupus is related to the cryoglobulin fraction of IC and might increase the risk of bacterial infection, especially deep infections caused by *S. aureus*, within the next two months. Circulating IC, which block contact between phagocytic receptors and opsonised bacteria, seem to be of importance, since they reduce host defence against protein A bearing bacteria; this is quite independent of the many other factors that have been considered as being more or less conducive to infection proneness in SLE patients. In fact infections in SLE patients are most frequently caused by *S. aureus* (unpublished data).^x

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